WEST Search History

Hide Items | Restore | Clear | Cancel

DATE: Friday, March 02, 2007

Hide?	Set Name	Query	Hit Count
	DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD; PLUR=YES; OP=OR		
☐	L1	cn=119-58-4	4294967295
	DB=TDBD,DWPI,JPAB,EPAB,USOC,USPT,PGPB; PLUR=YES; OP=OR		
☐	L2	(119-584)!	1
☐	L3	(119-58)!	42
	DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD; PLUR=YES; OP=OR		
☐	L4	l3 and (michler's near hydrol)	0
☐	L5	(michler's near hydrol)	374
☐	L6	(michlershydrol)	2
☐	L7	(michlers-hydrol)	0
☐	L8	(michler-hydrol)	0
☐	L9	(michler near hydrol)	374
☐	L10	bisdimethylamionbenzhydrol	0
☐	L11	dimethylamionbenzhydrol	0
☐	L12	dimethylaminobenzhydrol	14
☐	L13	bisdimethylaminobenzhydrol	3
☐	L14	bis-dimethylaminobenzhydrol	0
☐	L15	bis-dimethyl-aminobenzhydrol	0
☐	L16	bis-dimethyl-amino-benzhydrol	0
☐	L17	bis-dimethyl-amino-diphenyl-carbinol	0
☐	L18	bis-dimethyl-amino-diphenylcarbinol	0
☐	L19	bis-dimethyl-aminodiphenylcarbinol	0
☐	L20	bis-dimethylaminodiphenylcarbinol	0
☐	L21	bisdimethylaminodiphenylcarbinol	0
☐	L22	bis near dimethyl near amino near diphenyl near carbinol	0
☐	L23	bis near dimethylamino near diphenyl near carbinol	4
☐	L24	bis near dimethylamino near diphenylcarbinol	2
☐	L25	bis near dimethylaminodiphenylcarbinol	1
☐	L26	benzydrol near4 dimethylamino	0
☐	L27	benzydrol-dimethylamino	0
☐	L28	benzydrol near2 dimethylamino	0

☐	L29	benzhydrol near2 dimethylamino	81
☐	L30	benzhydrol near4 dimethylamino	90
☐	L31	benzhydrol-dimethylamino	0
☐	L32	benzhydrol near dimethylamino	79
☐	L33	bis near dimethylamino near phenyl near methanol	0
☐	L34	bis near dimethylamino near phenylmethanol	0
☐	L35	bis near dimethylaminophenylmethanol	0
☐	L36	dimethyl near aminophenylmethanol	0
☐	L37	dimethylamino near phenylmethanol	0
☐	L38	bis near dimethylamino near benzhydrol	70
☐	L39	l5 or l9 or l12 or l13 or l23 or l24 or l25 or l29 or l30 or l32 or l38	435
☐	L40	L39 and (ammonia or nh3 or nh-3)	206
☐	L41	L40 and ($device or apparat$ or $sensor or nanotube or nanoparticles or nanoparticle)	99
☐	L42	L39.clm. and (ammonia or nh3 or nh-3).clm.	3
☐	L43	L39.ti,ab,clm. and (ammonia or nh3 or nh-3).ti,ab,clm.	6
☐	L44	L39 same (ammonia or nh3 or nh-3) not l42 not l43	26
☐	L45	L39 same (ammonia or nh3 or nh-3) not l42 not l43	26
☐	L46	L39 and (ammonia or nh3 or nh-3).ti. not l42 not l43	0
☐	L47	L39 and (ammonia or nh3 or nh-3).ti,ab,clm. not l42 not l43	15
☐	L48	l41 not l42 not l43 not l44 not l45 not l46 not l47	87
☐	L49	l39 not l40	229
☐	L50	l39 near10 (ph or indicator)	6
☐	L51	l39 same indicator not l50	4
☐	L52	l39.ti. and (amide or amine or ammonia or nh3 or nh-3)	3

END OF SEARCH HISTORY

Enter a name, molecular formula or cas number ››more info

Find chemicals. 119-58-4 Search Search by structure Advanced Search

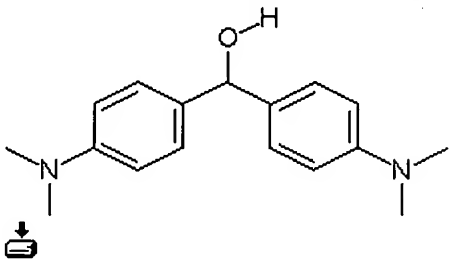
- 4,4'-Bis(dimethylamino) benzhydrol
- 4,4'-Bis(dimethylamino) diphenyl carbinol
- 4,4'-bis-(Dimethylamino) benzhydrol
- Michler's hydrol
- 4,4'-Bis(dimethylamino) benzhydrol = 4,4'-Bis (dimethylamino)diphenyl carbinol
- Benzhydrol 4-4' - Bis {Dimethylamino}
- 4,4'-Bis(dimethylamino) diphenylcarbinol
- bis(4-(dimethylamino)phenyl) methanol
- 4,4'-Bis(Dimethylamino) benzhydrol

RN: 119-58-4

MF: C17 H22 N2 O
C17H22N2O

MW: 270.37448

mp (°C): 98 - 105



IR: Show

3D model: Show

Hazard: XI

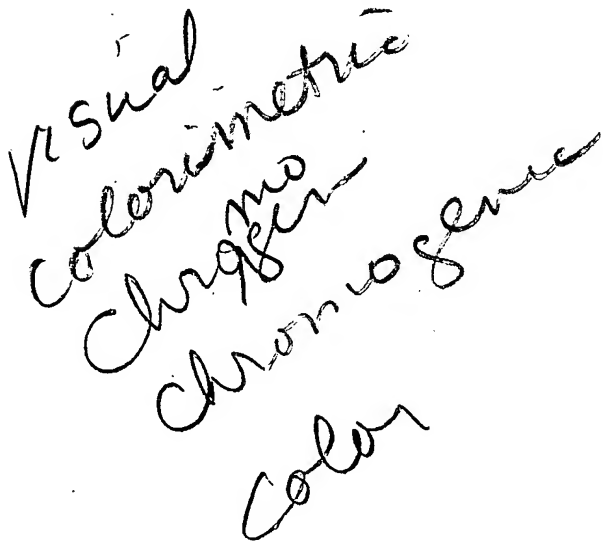
Risk: 36/37/38

Safety: 26 37/39

MSDS: EN

Search notes including all US Patent documents

The method exploits the known route for malodour generation in which protein is broken down by bacterial putrefaction into thiol containing compounds, as described by Grigor et al. in J. Dental Research 71, 1348, Abs. 347 (1992); ibid 72, 1347, Abs. 1347 and 1348 (1993). The compounds containing thiol groups are the precursors to the volatile sulphur compounds (hydrogen sulphide and methyl mercaptan). The experimental procedure involves the collection of saliva before, and 30 minutes after, use of the products being evaluated. The saliva samples are then incubated for 24 hours and the thiol concentration measured by the use of a colorimetric indicator (4,4'-bis(dimethylamino)-diphenyl carbinol). A measure of the reduction in the total thiol concentration in saliva is obtained for the test product. The extent of protein degradation that has occurred to generate thiols is taken as a predictor of the ultimate levels of the volatile products hydrogen sulphide and methyl mercaptan and hence the malodour potential of the saliva. The results are shown as the mean .DELTA. A R-SH relative to water controls.



Nanoparticles


US007052854B2

(12) **United States Patent**
Melker et al.

(10) **Patent No.: US 7,052,854 B2**
(45) **Date of Patent: May 30, 2006**

(54) **APPLICATION OF NANOTECHNOLOGY AND SENSOR TECHNOLOGIES FOR EX-VIVO DIAGNOSTICS**

(75) Inventors: **Richard J. Melker**, Gainesville, FL (US); **Ronald L. Hayes**, Gainesville, FL (US); **Ka-Wang Kevin Wang**, Gainesville, FL (US); **Donn Michael Dennis**, Gainesville, FL (US)

(73) Assignee: **University of Florida Research Foundation, Inc.**, Gainesville, FL (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 189 days.

(21) Appl. No.: **10/678,506**

(22) Filed: **Oct. 2, 2003**

(65) **Prior Publication Data**

US 2006/0040318 A1 Feb. 23, 2006

Related U.S. Application Data

(63) Continuation-in-part of application No. 10/345,532, filed on Jan. 16, 2003, and a continuation-in-part of application No. 10/154,201, filed on May 22, 2002, and a continuation-in-part of application No. 10/274,829, filed on Oct. 21, 2002.

(60) Provisional application No. 60/292,962, filed on May 23, 2001.

(51) **Int. Cl.**
G01N 33/53 (2006.01)

(52) **U.S. Cl.** **435/7.1**; 435/4; 435/6; 436/501; 436/535; 422/68.1; 422/82.01; 422/82.02; 422/98

(58) **Field of Classification Search** 435/7.1, 435/6, 182, 4; 436/535, 501; 73/23.2, 24.06; 422/68.1, 82.01, 82.02, 98; 702/19, 22
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

3,567,029	A	3/1971	Quame
3,608,546	A	9/1971	Shinn
3,649,199	A	3/1972	Littlejohn
3,792,272	A	2/1974	Harte et al.
3,877,291	A	4/1975	Hoppesch et al.
3,951,607	A	4/1976	Fraser
3,955,926	A	5/1976	Fischer
4,150,670	A	4/1979	Jewett et al.
4,202,352	A	5/1980	Osborn
4,215,409	A	7/1980	Strowe
4,312,228	A	1/1982	Wohltjen
4,314,564	A	2/1982	Albarda
4,334,540	A	6/1982	Preti et al.
4,346,584	A	8/1982	Boehringer
4,349,626	A	9/1982	Labows et al.
4,361,026	A	11/1982	Muller et al.
4,399,686	A	8/1983	Kindlund et al.
4,432,226	A	2/1984	Dempster
4,456,014	A	6/1984	Buck et al.
4,534,360	A	8/1985	Williams
4,734,777	A	3/1988	Okino et al.
4,735,777	A	4/1988	Mitsui et al.
4,772,559	A	9/1988	Preti et al.
4,796,639	A	1/1989	Snow et al.
4,868,545	A	9/1989	Jones
4,895,017	A	1/1990	Pyke et al.
4,938,928	A	7/1990	Koda et al.
4,992,244	A	2/1991	Grate
5,003,985	A	4/1991	White et al.
5,034,192	A	7/1991	Wrighton et al.
5,042,501	A	8/1991	Kenny et al.
5,060,506	A	10/1991	Douglas
5,071,770	A	12/1991	Kolesar, Jr.
5,081,871	A	1/1992	Glaser
5,082,630	A	1/1992	Partin et al.
5,094,235	A	3/1992	Westenskow et al.
5,111,827	A	5/1992	Rantala
5,137,692	A	8/1992	Fritz
5,145,645	A	9/1992	Zakin et al.
5,167,972	A	12/1992	Greenberg et al.
5,179,027	A	1/1993	Fisher
5,252,292	A	10/1993	Hirata et al.
5,296,706	A	3/1994	Braig et al.
5,303,575	A	4/1994	Brown et al.
5,317,156	A	5/1994	Cooper et al.
5,325,704	A	7/1994	Mariani et al.
5,351,522	A	10/1994	Lura
5,361,771	A	11/1994	Craine et al.

(Continued)

FOREIGN PATENT DOCUMENTS

DE 19607646 A1 9/1997

(Continued)

OTHER PUBLICATIONS

Hong C. et al., "Carbon Nanotube-Enhanced Electrochemical DNA Biosensor for DNA Hybridization Detection" (2003), *Anal. Bioanal. Chem.*, 375:287-293.

(Continued)

Primary Examiner—Long V. Le
Assistant Examiner—Jacqueline A. DiRamio
(74) *Attorney, Agent, or Firm*—Saliwanchik, Lloyd & Saliwanchik

(57) **ABSTRACT**

Systems and methods for the ex vivo diagnostic analysis of samples of bodily fluids, including exhaled breath and blood. The present invention uses nanostructure-based assemblies in combination with sensor technology to provide an efficient and accurate means for identifying the presence of a target analyte/biomarker in a sample of bodily fluid. In a preferred embodiment, the nanostructure-based assemblies of the present invention include detecting means such as RNA oligonucleotide chains or "apparatus" and releasable surrogate markers such as DMSO.

11 Claims, No Drawings

U.S. PATENT DOCUMENTS

5,409,839	A		4/1995	Balestrieri et al.
5,425,374	A		6/1995	Ueda et al.
5,447,165	A		9/1995	Gustafsson
5,453,359	A		9/1995	Gargan et al.
5,465,608	A		11/1995	Lokshin et al.
5,466,700	A		11/1995	Batenhorst et al.
5,482,601	A		1/1996	Ohshima et al.
5,495,744	A		3/1996	Ueda et al.
5,501,212	A		3/1996	Psaros
5,528,924	A		6/1996	Wajid et al.
5,547,878	A		8/1996	Kell
5,558,083	A		9/1996	Bathe et al.
5,560,352	A		10/1996	Heim et al.
5,571,401	A	*	11/1996	Lewis et al. 205/787
5,573,005	A		11/1996	Ueda et al.
5,573,955	A		11/1996	Khanna et al.
5,605,612	A		2/1997	Park et al.
5,634,517	A		6/1997	Linden et al.
5,645,072	A		7/1997	Thrall et al.
5,716,852	A		2/1998	Yager et al.
5,756,879	A		5/1998	Yamagishi et al.
5,771,890	A		6/1998	Tamada
5,776,783	A		7/1998	Kell
5,783,154	A		7/1998	Althainz et al.
5,783,449	A		7/1998	Kuznetsov
5,795,787	A		8/1998	Silkoff et al.
5,801,297	A		9/1998	Mifsud et al.
5,826,577	A		10/1998	Perroz et al.
5,830,412	A		11/1998	Kimura et al.
5,861,254	A		1/1999	Schneider et al.
5,866,434	A	*	2/1999	Massey et al. 436/526
5,891,398	A		4/1999	Lewis et al.
5,900,552	A		5/1999	Chu et al.
5,918,257	A		6/1999	Mifsud et al.
5,925,014	A		7/1999	Teeple, Jr.
5,928,167	A		7/1999	Wagner et al.
5,932,877	A		8/1999	Braig et al.
5,945,069	A		8/1999	Buehler
5,950,630	A		9/1999	Portwood et al.
5,954,685	A		9/1999	Tierney
5,958,896	A		9/1999	Renshaw et al.
5,962,335	A		10/1999	Katzman
5,971,937	A		10/1999	Ekstrom
5,996,586	A		12/1999	Phillips
6,007,775	A		12/1999	Yager
6,010,459	A		1/2000	Silkoff et al.
6,025,200	A		2/2000	Kaish et al.
6,057,162	A		5/2000	Rounbehler et al.
6,063,243	A		5/2000	Zettl et al.
6,067,167	A		5/2000	Atkinson et al.
6,074,345	A		6/2000	Van Oostrom et al.
6,085,576	A		7/2000	Sunshine et al.
6,094,681	A		7/2000	Shaffer et al.
6,097,485	A		8/2000	Lievan
6,120,443	A		9/2000	Cohen-Laroque
6,131,571	A		10/2000	Lampotang et al.
6,136,801	A		10/2000	Kell
6,153,147	A		11/2000	Craig
6,180,414	B1		1/2001	Katzman
6,186,977	B1		2/2001	Andrews et al.
6,190,858	B1		2/2001	Persaud et al.
6,203,814	B1		3/2001	Fisher et al.
6,216,690	B1		4/2001	Keitel et al.
6,221,026	B1		4/2001	Phillips
6,234,006	B1		5/2001	Sunshine et al.
6,237,397	B1		5/2001	Shinar et al.
6,244,096	B1		6/2001	Lewis et al.
6,248,078	B1		6/2001	Risby et al.
6,251,082	B1		6/2001	Rayburn
6,261,783	B1		7/2001	Jayasena et al.
6,264,913	B1		7/2001	Wagner
6,277,081	B1		8/2001	Susi et al.
6,283,953	B1		9/2001	Ayer et al.
6,303,316	B1		10/2001	Kiel et al.
6,305,212	B1		10/2001	Drzewiecki
6,312,390	B1		11/2001	Phillips
6,319,724	B1		11/2001	Lewis et al.
6,328,708	B1		12/2001	Georgieff
6,341,520	B1		1/2002	Satoh et al.
6,363,772	B1		4/2002	Berry
6,387,329	B1		5/2002	Lewis et al.
6,399,302	B1		6/2002	Lannigan et al.
6,416,479	B1		7/2002	Seidman
6,455,319	B1		9/2002	Lewis et al.
6,467,333	B1	*	10/2002	Lewis et al. 73/31.05
6,479,019	B1		11/2002	Goldstein et al.
6,495,824	B1		12/2002	Atkinson
6,511,453	B1		1/2003	Georgieff
6,558,626	B1		5/2003	Aker et al.
6,589,727	B1		7/2003	Klenerman et al.
6,597,438	B1		7/2003	Cabuz et al.
6,598,459	B1	*	7/2003	Fu 73/23.34
6,599,253	B1		7/2003	Baum et al.
6,599,281	B1		7/2003	Struys et al.
6,620,800	B1		9/2003	Roberts, II
6,631,333	B1		10/2003	Lewis et al.
6,680,377	B1		1/2004	Stanton et al.
6,727,075	B1		4/2004	Fitzgerald et al.
6,755,783	B1		6/2004	Cosentino et al.
2001/0021815	A1		9/2001	Katzman et al.
2001/0041366	A1		11/2001	Lewis et al.
2001/0046674	A1		11/2001	Ellington
2001/0050228	A1		12/2001	Jaeger
2001/0055544	A1		12/2001	Copp
2002/0007249	A1		1/2002	Cranley et al.
2002/0007687	A1		1/2002	Zimmermann et al.
2002/0014236	A1		2/2002	Dittmann et al.
2002/0017300	A1		2/2002	Hickle et al.
2002/0034757	A1		3/2002	Cubicciotti
2002/0068295	A1		6/2002	Madou et al.
2002/0173729	A1		11/2002	Viertio-Oja et al.
2002/0177232	A1		11/2002	Melker et al.
2003/0004426	A1		1/2003	Melker et al.
2003/0008407	A1		1/2003	Fu
2003/0059820	A1		3/2003	Vo-Dinh
2003/0087239	A1		5/2003	Stanton et al.
2003/0119065	A1		6/2003	Lin et al.
2003/0139681	A1		7/2003	Melker et al.
2003/0216660	A1		11/2003	Ben-Oren et al.
2004/0027246	A1		2/2004	Aguglia
2004/0101477	A1		5/2004	Leyland-Jones
2005/0065446	A1		3/2005	Talton

FOREIGN PATENT DOCUMENTS

DE	29902593		8/1999
EP	0 370 151	A1	5/1990
EP	0 979 997	A1	2/2000
GB	829409	A	3/1960
GB	2 309 166	A	7/1997
GB	2 329 245	A	3/1999
JP	08313407	A	11/1996
JP	09196915	A	7/1997
WO	WO 87/02773	A1	5/1987
WO	WO 92/10749	A1	6/1992
WO	WO 95/08113	A1	3/1995
WO	WO 95/31718	A1	11/1995
WO	WO 98/57145		12/1998
WO	WO 99/12471	A3	3/1999
WO	WO 99/66304	A1	12/1999
WO	WO 00/25108	A1	5/2000
WO	WO 00/67820	A1	11/2000
WO	WO 00/79243	A1	12/2000
WO	WO 01/34024	A1	5/2001

WO	WO 01/93743 A2	12/2001
WO	WO 02/079514	10/2002
WO	WO 03/016901	2/2003
WO	WO 03/045473 A1	6/2003
WO	WO 2004/065404	8/2004

OTHER PUBLICATIONS

Pantarotto D. et al., "Synthesis, Structural Characterization, and Immunological Properties of Cabon Nanotubes Functionalized with Peptides" (2003), *J. Am. Chem. Soc.*, 125:6160-6164.

Ballantine, DS. et al. "Surface Acoustic Wave Devices for Chemical Analysis," *Anal. Chem.* (1989), vol. 61, No. 11, pp. 704A-712A.

Brody, EN. et al. "Aptamers as Therapeutic and Diagnostic Agents," *Reviews in Molecular Biotechnology* (2000), vol. 74, pp. 5-13.

Brody et al. "The use of Aptamers in Large Arrays for Molecular Diagnostics," *Molecular Diagnosis* (1999), vol. 4, No. 4, pp. 3811-388.

Chandiok, S. et al. "Screening for Bacterial vaginosis: A Novel Application of Artificial Nose Technology," *Journal of Clinical Pathology* (1997), vol. 50, pp. 790-791.

Fang, M. et al. "Detection of Organic Chemicals by SAW Sensor Array," *Sensors and Actuators* (1999), vol. B56, pp. 155-157.

Fischer et al. "A man-portable chemical sniffer utilizing Novel Fluorescent polymers for detection of ultra-trace concentrations of explosives emanating from landmines," *Nomadics Inc.* (2000), pp. 1-10.

Ganga-Zandzou, P.S. et al. "A 13C-urea breath test in children with *Helicobacter pylori* infection: validity of the use of a mask to collect exhaled breath sample," *Acta. Paediatr.* (2001), vol. 90, pp. 232-233.

Grate, JW et al. "Determination of Partition Coefficients from Surface Acoustic Wave Vapor Senor Responses and Correlation with Gas-Liquid Chromatographic Partition Coefficients," *Anal. Chem.* (1998), vol. 60, pp. 869-875.

Groves, W. et al. "Analyzing organic vapors in exhaled breath using a surface acoustic wave sensor array with preconcentration: Selection and characterization of the preconcentrator absorbent," *Analytica Chimica Acta* (1998), vol. 371, pp. 131-143.

Hanson, C.W. et al. "The use of a novel 'Electronic Nose' to diagnose the presence of intrapulmonary infection," *Anesthesiology* (1997), vol. 87, No. 3A, abstract A269.

Liebich et al. "Volatile Substances in Blood Serum: a Profile Analysis and Quantitative Determination," *Journal of Chromatography* (1977), vol. 142, pp. 505-516.

Mueller et al. "Experience in mass spectrometric identification in acute poisoning," *Beitr. Diagn. Ther, Akuter. Intox., Vortr. Symp. 4th* (1982), pp. 126-134, Abstract Only.

Parry, A.D. et al. "Leg Ulcer Odour Detection Identifies β-haemolytic *Streptococcal* Infection," *Journal of Wound Care* (1995). vol 4, No. 9, pp. 404-406.

Paviou and Turner. "Sniffing out the truth: Clinical Diagnosis Using the Electronic Nose," *Clin. Chem. Lab. Med.* (2000), vol. 38, No. 2, pp. 99-112.

Perri, F. "Diagnosis of *Helicobacter pylori* infection: which is best? The urea breath test," *Dig. Liver. Dis.* (2000), vol. 32, Supp. 3, pp. S196-198.

Rogers et al. "Fiber-optic biosensors based on total internal-reflection fluorescence," *American Chemical Society* (1992), Ch. 13, pp. 165-173.

Stojanovic et al. "Aptamer—based Folding Fluorescent Sensor for Cocaine," *J. Am. Chem. Soc.* (2001), vol. 123, pp. 4928-4931.

Tracqui, A. et al. "Systematic Toxicological Analysis Using HPLC/DAD," *Journal of Forensic Sciences* (1995), vol. 40, No. 2, pp. 254-262.

Wohltjen, H. et al. "Vapor Detection with Surface Acoustic Wave Microsensors," *Chemical Sensors and Microinstrumentation* (1989), pp. 157-175.

U.S. Appl. No. 09/708,789, filed Nov. 8, 2000, Lampotang et al.

Dickinson, T. A. et al., "Current Trends in 'Artificial-Nose' Technology," *Tib Tech*, 1998, 16:250-258.

Frauendorf et. al., "Detection of Small Organic Analytes by Fluorescing Molecular Switches," *Bioorganic & Medicinal Chemistry 9*, (2001), pp. 2521-2524.

Fujita et. al., "A Simple Method for Dectecting Plasma Propofol," *Anesth. Analog.*, 2000, 90:1452-1454.

Hammon III, W. S. et al., "Forensic GPR: Finite-Difference Simulations of Responses From Buried Human Remains," *Journal of Applied Geophysics*, (2000), 45:171-186.

Huang et. al., "Depth of Anesthesia Estimating & Propofol Delivery System," Aug. 1, 1996, .rpi.edu/~royr/roy_descpt.html.

Jayasena, "Aptamers: An Emerging Class of Molecules That Rival Antibodies in Diagnositcs," *Clinical Chemistry*, 1999, 45(9):1628-1650.

Kenny, "Target-Controlled Infusions—Pharmacokinetic and Pharmacodynamic Variations," .anaesthesiologie.med.uni-erlangen.de/esctaic97/a_kenny.htm.

Kuipers et al., "First-pass Lung Uptake and Pulmonary Clearance of Propofol," *Anesthesiology*, (1999), 91:1780-1787.

Miller III, E. R. et al., "Association Between Cigarette Smoking and Lipid Peroxidation in a Controlled Feeding Study," *Circulation*, (1997), 96(4):1097-1101.

Phillips, M., "Breath Tests in Medicine," *Scientific American*, 1992, pp. 52-57.

Pilar Kraman, "Prescription Drug Diversion," *Trends Alert* provided by the Council of State Government at csg.org (Apr. 2004).

Stuart, B. H. et al., "Studies of Adipocere Using Diffuse Reflectance Infrared Spectroscopy," *Vibrational Spectroscopy*, 24:233-242, (2000).

Stubbs, D. D. et al., "Investigation of Cocaine Plumes Using Surface Acoustic Wave Immunoassay Sensors," *Annal. Chem.*, 75:6231-6235, (2003).

U.S. Food and Drug Administration, "FDA White Paper, Protecting the Public Health: FDA Pursues and Aggressive Enforcement Strategy," fda.gov/oc/whitepapers/enforce.html (Jun. 30, 2003).

U.S. Food and Drug Administration, "New FDA Initiative to Combat Counterfeit Drugs," fda.gov/oc/initiatives/counterfeit/backgrounder.html (Jul. 2, 2004).

United States Department of Justice, "Review of the Drug Enforcement Administration's (DEA) Control of the Diversion of Controlled Pharmaceuticals," Report No. I-2002-010 www.usdoj.gov/oig/inspection/DEA/0210/background.htm (Sep. 2002).

Vass, A., "Beyond the Grave—Understanding Human Decomposition," *Microbiology Today*, Nov. 2001, 28:190-192.

Vass, A. et al., "Decomposition Chemistry of Human Remains: A New Methodology for Determining the Postmortem Interval," *J. Forensic Sci.*, (2002), 47(3):542-553.

Vass, A. et al., "Detection of Buried Human Remains Using Bioreporter Fluorescence," U.S. Dept. of Energy Report, Y/NSP-726 (2001).

* cited by examiner

APPLICATION OF NANOTECHNOLOGY AND SENSOR TECHNOLOGIES FOR EX-VIVO DIAGNOSTICS

CROSS-REFERENCE TO RELATED APPLICATION

This application is a continuation-in-part of co-pending U.S. application Ser. No. 10/154,201, filed May 22, 2002; which claims the benefit of U.S. application Ser. No. 60/292,962, filed May 23, 2001. This application is also a continuation-in-part of co-pending U.S. application Ser. No. 10/274,829, filed Oct. 21, 2002; and a continuation-in-part of co-pending U.S. application Ser. No. 10/345,532, filed Jan. 16, 2003, all of which are hereby incorporated by reference herein in their entirety, including any figures, tables, or drawings.

GOVERNMENT SUPPORT

The subject matter of this application has been supported in part by a research grant from the National Science Foundation (Grant Number NSF: EEC 02-10580). Accordingly, the government may have certain rights in this invention.

BACKGROUND OF THE INVENTION

There is a great need for the development of efficient and accurate systems for the diagnosis of a variety of medical conditions, disorders, and diseases. A means for rapid and accurate analysis and diagnosis of ex vivo bodily fluid samples at the point of care is particularly desirable. This requires an effective means for identifying in a patient the presence of specific chemical and/or biological agents. including, but not limited to, nucleic acids, proteins, illicit drugs, toxins, pharmaceuticals, carcinogens, poisons, allergens, and infectious agents.

Current methods of detecting such chemical or biological agents entail extraction of a sample into organic solvents, followed by analysis using stand-alone analytical systems such as gas-liquid chromatography and/or mass spectroscopy. These methods are time-consuming and often expensive. Moreover, certain analytes/biomarkers of interest (i.e., nucleic acids, carcinogens, or toxins) are not readily detected by standard chemical tests utilized in a typical clinical physician's office or even in hospital departments. Further, on-site test devices for accurate analyte/biomarker detection are not presently available. The development of a biosensor device that could accurately and efficiently detect/screen for chemical and biological agents in bodily fluid samples would therefore provide a significant cost and time benefit.

Three recent advancements in medicine are particularly germane to expanding the potential of detecting chemical and/or biological agents, especially with regard to the diagnosis of disease: nanotechnology, biodetectors (biosensors), and the identification of biomarkers for specific diseases and/or conditions. Nanotechnology, such as nanoparticles, offers many advantages when used for applications such as sensor technology for detecting chemical agents. For example, nanoparticle-bound surfaces can form a sensor effective in detecting a target chemical agent.

The term "biodetectors" or "biosensors" relates to the use of naturally occurring and/or synthetic compounds as highly specific and extraordinarily sensitive detectors of various types of molecules and markers of disease. Naturally-occurring compounds such as antibodies have been used to provide molecular recognition for a wide variety of target molecules in diagnostic assays. Alternatively, synthetic compounds have been manufactured that mimic naturally-occurring mechanisms of DNA, RNA, and protein synthesis in cells to facilitate the detection of target chemical or biological agents.

Aptamers have recently been identified as potentially effective biosensors for molecules and compounds of scientific and commercial interest (see Brody, E. N. and L. Gold, "Aptamers as therapeutic and diagnostic agents," *J. Biotechnol.*, 74(1):5–13 (2000) and Brody et al., "The use of aptamers in large arrays for molecular diagnostics," *Mol. Diagn.*, 4(4):381–8 (1999)). For example, aptamers have demonstrated greater specificity and robustness than antibody-based diagnostic technologies. In contrast to antibodies, whose identification and production completely rest on animals and/or cultured cells, both the identification and production of aptamers takes place in vitro without any requirement for animals or cells.

Aptamer synthesis is potentially far cheaper and reproducible than antibody-based diagnostic tests. Aptamers are produced by solid phase chemical synthesis, an accurate and reproducible process with consistency among production batches. An aptamer can be produced in large quantities by polymerase chain reaction (PCR) and once the sequence is known, can be assembled from individual naturally occurring nucleotides and/or synthetic nucleotides. Aptamers are stable to long-term storage at room temperature, and, if denatured, aptamers can easily be renatured, a feature not shared by antibodies. Furthermore, aptamers have the potential to measure concentrations of ligand in orders of magnitude lower (parts per trillion or even quadrillion) than those antibody-based diagnostic tests. These inherent characteristics of aptamers make them attractive for diagnostic applications.

A number of "molecular beacons" (often fluorescence compounds) can be attached to aptamers to provide a means for signaling the presence of and quantifying a target chemical or biological agent. For instance, an aptamer specific for cocaine has recently been synthesized (Stojanovic; M. N. et al., "Aptamer-based folding fluorescent sensor for cocaine," *J. Am. Chem. Soc.*, 123(21):4928:31 (2001)). A fluorescence beacon, which quenches when cocaine is reversibly bound to the aptamer is used with a photodetector to quantify the concentration of cocaine present. Aptamer-based biosensors can be used repeatedly, in contrast to antibody-based tests that can be used only once.

Of particular interest as a beacon are amplifying fluorescent polymers (AFP). AFPs with a high specificity to TNT and DNT have been developed. It has been noted that a detector based on AFP technology, with high specificity to TNT and DNT, can also detect propofol, an intravenous anesthetic agent, in extremely low concentrations. The combination of AFP and aptamer technologies holds the promise of robust, reusable biosensors that can detect compounds in minute concentrations with high specificity.

The term "biomarker" refers to a biochemical in the body that has a particular molecular trait to make it useful for diagnosing a condition, disorder, or disease and for measuring or indicating the effects or progress of a condition, disorder, or disease. For example, common biomarkers found in a person's bodily fluids (i.e., breath or blood), and the respective diagnostic conditions of the person providing such biomarkers include, but are not limited to, acetaldehyde (source: ethanol; diagnosis: intoxication), acetone (source: acetoacetate; diagnosis: diet; ketogenic/diabetes), ammonia

(source: deamination of amino acids; diagnosis: uremia and liver disease), CO (carbon monoxide) (source: CH_2Cl_2, elevated % COH; diagnosis: indoor air pollution), chloroform (source: halogenated compound), dichlorobenzene (source: halogenated compounds), diethylamine (source: choline; diagnosis: intestinal bacterial overgrowth), H (hydrogen) (source: intestines; diagnosis: lactose intolerance), isoprene (source: fatty acid; diagnosis: metabolic stress), methanethiol (source: methionine; diagnosis: intestinal bacterial overgrowth), methylethylketone (source: fatty acid; diagnosis: indoor air pollution/diet), O-toluidine (source: carcinoma, metabolite; diagnosis: bronchogenic carcinoma), pentane sulfides and sulfides (source: lipid peroxidation; diagnosis: myocardial infarction), H_2S (source: metabolism; diagnosis: periodontal disease/ovulation), MeS (sucrose: metabolism; diagnosis: cirrhosis), and Me_2S (source: infection; diagnosis: trench mouth).

Mechanisms of drug metabolism are extremely complex and are influenced by a number of factors including competitive binding on protein and red blood cells with other molecules, enzymatic activity, particularly in the liver, protein, and red blood cell concentration and a myriad of other factors. Currently, very little technology is available that can measure drug concentration in a patient in real-time, especially at the point of care, and thereby allow convenient determination of pharmacokinetics and pharmacodynamics of multiple compounds in real-time.

Accordingly, there are a number of medical conditions that can be monitored by detecting and/or measuring biomarkers present in a person's breath (including breath condensates or aerosolized particles) or other bodily fluids. While there has been technology generated towards the synthesis and use of aptamers and other multimolecular devices such as biosensors, very little technology exists to address the use of aptamers, or other biodetectors, in combination with nanoparticles to form sensors for the ex vivo diagnosis of disease and/or detection of a naturally occurring or synthetic compounds. It is therefore desirable to provide a low-cost means for accurately and timely detecting and/or measuring the presence of metabolites in a person's bodily fluids in low concentrations.

BRIEF SUMMARY

The present invention provides unique systems and methods for the ex vivo detection of analytes/biomarkers of interest in samples of bodily fluids. The invention comprises a nanostructure-based assembly that is applied to bodily fluid samples collected from a patient. In accordance with the present invention, a nanostructure-based assembly contains (a) a nanoparticle, which includes (b) a means for detecting a target analyte/biomarker (i.e., aptamers, antibodies); and (c) at least one surrogate marker. These components can be attached to any surface of the nanoparticle.

In operation, a sample of bodily fluid is collected from a patient and the nanostructure-based assembly of the invention is applied to the sample. When the detecting means detects a target analyte/biomarker, the surrogate marker is released from the nanoparticle. Because surrogate markers are released from nanoparticles only in the presence of a target analyte/biomarker, detection of surrogate markers provides notice of the presence of the target analyte/biomarker in the patient and consequently, allows diagnosis of the specific condition, disorder, or disease associated with the target analyte/biomarker. Not only do surrogate markers signal the presence of the target analyte/biomarker, but they also provide a means for quantifying the concentration of the analyte/biomarker of interest present in the sample of bodily fluid.

In accordance with the present invention, the detecting means includes well-known biodetectors or biosensors. Such biodetectors to biosensors include naturally occurring and/or synthetic compounds having high specificity and sensitivity to chemical and/or biological compounds of interest. Suitable biodetectors or biosensors of the invention include, but are not limited to, antibodies, proteins, and aptamers.

The surrogate marker of the invention is a compound that is readily detectable in bodily fluid samples. In preferred embodiments, the surrogate marker is a volatile compound (i.e., dimethyl sulfoxide—DMSO).

According to the invention, a sample of bodily fluid includes, but is not limited to, exhaled breath (including cough, sneeze), blood, urine, sweat, mucous, semen, bile, feces, saliva, lymph fluid, blood plasma, amniotic fluid, glandular fluid, sputum, and cerebral spinal fluid. The bodily fluid sample is analyzed for the presence of the surrogate marker, which indicates the presence of the target analyte/biomarker in the patient and consequently, allows for the diagnosis of the condition, disease, or disorder associated with the target analyte/biomarker.

For analysis of bodily fluid samples to detect the presence of the surrogate marker, sensor technology is applied in accordance with the present invention. Contemplated sensor technology includes, but is not limited to, previously disclosed sensor technology such as semiconductor gas sensor technology, conductive polymer gas sensor technology, surface acoustic wave gas sensor technology, and immunoassays.

The results from the sensor technology analysis of the bodily fluid samples are optionally provided to the user (or patient) via a reporting means. In one embodiment, the sensor technology includes the reporting means. Contemplated reporting means include a computer processor linked to the sensor technology in which electronic or printed results are provided. Alternatively, the reporting means can include a digital display panel, transportable read/write magnetic media such as computer disks and tapes which can be transported to and read on another machine, and printers such as thermal, laser or ink-jet printers for the production of a printed report. The reporting means can provide the results to the user (or patient) via facsimile, electronic mail, mail or courier service, or any other means of safely and securely sending the report to the patient. Interactive reporting means are also contemplated by the present invention, such as an interactive voice response system, interactive computer-based reporting system, interactive telephone touch-tone system, or other similar system. The report provided to the user (or patient) may take many forms, including a summary of analyses performed over a particular period of time or detailed information regarding a particular bodily fluid sample analysis. Results may also be used to populate a financial database for billing the patient, or for populating a laboratory database or a statistical database.

In one embodiment, the nanoparticle of the nanostructure-based assembly has a hollow body defining an inner void, which contains the surrogate marker. Release of the surrogate marker is controlled by the means for detecting the target analyte/biomarker. In certain embodiments, the detecting means is attached to an end-cap, which covers an opening to the inner void. In other embodiments, the detecting means is attached to the surface of the nanoparticle. The detecting means is designed to undergo a conformational

change upon detecting the target analyte/biomarker to affect the release of the surrogate marker from the nanoparticle. With reference to those embodiments in which the detecting means is attached to an end-cap, the conformational change of the detecting means in the presence of a target analyte/biomarker causes the end-cap to detach from the nanoparticle and release the surrogate marker.

In a related embodiment, the detecting means is attached to a surface of the nanoparticle. The detecting means can localize the nanostructure-based assembly to the target analyte/biomarker. When in the presence of the target analyte/biomarker, the controlled release of the surrogate marker is accomplished by the release of an end-cap, which is attached to the nanoparticle via chemically labile bonds. Preferably, the bonds attaching the end-cap to the nanoparticle are sensitive to the target analyte/biomarker.

Alternatively, the surrogate markers may be directly attached to a surface of the nanoparticle via chemically labile bonds that are sensitive to the target analyte/biomarker. Thus, when the nanostructure-based assembly is localized to the target analyte/biomarker by the detecting means, the surrogate markers are released without the need for an end-cap.

Yet another embodiment provides a solid nanoparticle that has the detecting means is attached to the outside surface of the nanoparticle and the surrogate marker is attached to the detecting means. In the presence of a target analyte/biomarker, the detecting means undergoes a conformational change to cause the surrogate marker to be detectable to the user. For example, the detecting means/surrogate marker of the invention can include aptamer-based molecules beacons.

In a preferred embodiment, nanoparticles are in the form of nanotubes, which are defined by a hollow tubular body with an inner void, which contains a surrogate marker. The hollow tubular body has a first end and a second end. The first end of the tubular body is open and a first end-cap bound to a detecting means is positioned over the first open end to close the first end. The second end of the tube is closed or similarly capped as the first end. Preferably, the detecting means is an aptamer.

The advantages of the invention are numerous. First and foremost, for healthcare personnel, the invention provides a method that can readily diagnose a patient's condition (or disorder/disease) based on a small sample of the patient's bodily fluid. Second, the invention is inexpensive and has broad medical applications for detecting a wide range of compounds (including licit and illicit drugs) in samples of bodily fluids.

For example, using the systems and methods of the present invention, emergency room personnel can quickly and effectively determine if someone is suffering from traumatic brain injury (TBI). By mixing nanostructure-based assemblies of the present invention to an ex vivo sample of the injured patient's blood or cerebral spinal fluid and applying sensor technology to the mixture, the present invention can accurately assess the presence and concentration of αII-spectrin breakdown products (biomarkers of TBI) for which the nanostructure-based assemblies are programmed to detect. A resulting advantage of the ability to rapidly detect TBI through a simple and efficient system is the ability to timely treat TBI.

In another embodiment, the nanostructure-based assemblies are designed to detect a protein, prostate specific antigen (PSA), which is produced by prostate cancers. In accordance with the present invention, a detecting means is designed that is specific for PSA (i.e., PSA-aptamer). The detecting means is attached to a surface of the nanoparticle. Also included on or within the nanoparticle is at least one surrogate marker (i.e., DMSO).

To test for the presence of prostate cancer, or a recurrence of prostate cancer, a nanostructure-based assembly designed to detect PSA is mixed with an ex-vivo sample of a patient's bodily fluid (i.e., blood). Where PSA is present in the bodily fluid sample, the PSA interacts with the detecting means, which affects the release of the surrogate marker for detection. Any one of a number of previously disclosed sensor technologies is then used to detect the surrogate marker, which indicates the presence and concentration of PSA in the bodily fluid sample.

Ex vivo analysis of bodily fluids utilizing methods disclosed herein can be applied to a wide range of point of care (POC) diagnostic tests. For example, potential applications include detection of licit and illicit drugs, detection of a wide range of biomarkers related to specific diseases, and detection of any other compounds that appear in bodily fluids. These tests can be highly quantitative because the quantity of surrogate markers released/detectable is proportional to the quantity of a target analyte/biomarker present in a sample of bodily fluid.

Moreover, analysis of bodily fluid samples using the method of the present invention can enable timely interventions for time-sensitive conditions or diseases. For example, it is known that isoprostane levels increase in cerebral spinal fluid and blood after TBI. If isoprostane is readily detectable in bodily fluids (i.e., blood, cerebral spinal fluid) using an isoprostane specific nanostructure-based assembly of the present invention, it can be possible to not only diagnose TBI in a patient but also to evaluate the efficacy of interventions in real-time for TBI. In addition, the method of the present invention can also evaluate pharmacodynamics and pharmacokinetics for drug interventions in individuals.

DETAILED DISCLOSURE

The present invention is directed to the efficient, timely, and accurate analysis of a sample of a patient's bodily fluids to detect and/or quantify analytes/biomarkers indicative of conditions, disorders, or diseases such as intoxication, cancer, cardiac disease, drug abuse, renal failure, liver disease, or diabetes. The systems and methods of the invention use nanostructure-based assemblies that contain a nanoparticle, a means for detecting a target analyte/biomarker, and a surrogate marker. Commonly available sensor technology is used by the present invention to detect the presence of a surrogate marker released from a nanostructure-based assembly in a sample of bodily fluid.

In operation, nanostructure-based assemblies of the invention are mixed with an ex vivo bodily fluid sample collected from a patient. Surrogate markers are generally released into the patient when nanostructure-based assemblies are in the presence of target analytes/biomarkers. Specifically, bioactive interaction between the biodetector/biosensor and the target analyte/biomarker affect the release of the surrogate marker from the nanoparticle. Advantageously, the concentration of the released surrogate marker is proportional to the amount of analyte/biomarker present in the bodily fluid sample, which can be measured using quantitative sensor technology known in the art.

Definition

Unless otherwise stated, the following terms used in the specification and claims have the meanings given below:

The term "aptamer," as used herein, refers to a non-naturally occurring oligonucleotide chain that has a specific action on a target analyte/biomarker of interest. A specific action includes, but is not limited to, binding the target analyte/biomarker, catalytically changing the target analyte/biomarker, and reacting with the target analyte/biomarker in a way which modifies/alters the analyte/biomarker or the functional activity of the analyte/biomarker. The aptamers of the invention preferably specifically bind to a target analyte/biomarker and/or react with the target analyte/biomarker in a way which modifies/alters the analyte/biomarker or the functional activity of the analyte/biomarker.

Aptamers include nucleic acids that are identified from a candidate mixture of nucleic acids. In a preferred embodiment, aptamers include nucleic acid sequences that are substantially homologous to the nucleic acid ligands isolated by the SELEX method. Substantially homologous is meant a degree of primary sequence homology in excess of 70%, most preferably in excess of 80%.

The "SELEX" methodology, as used herein, involves the combination of selected nucleic acid ligands, which interact with a target analyte/biomarker in a desired action, for example binding to a protein, with amplification of those selected nucleic acids. Optional iterative cycling of the selection/amplification steps allows selection of one or a small number of nucleic acids, which interact most strongly with the target analyte/biomarker from a pool, which contains a very large number of nucleic acids. Cycling of the selection/amplification procedure is continued until a selected goal is achieved. The SELEX methodology is described in the following U.S. patents and patent applications: U.S. patent application Ser. No. 07/536,428 and U.S. Pat Nos. 5,475,096 and 5,270,163.

The term "indicator aptamers," as used herein, refers to aptamers to which molecular beacons are attached, such as those described in U.S. Pat. Nos. 6,399,302 and 5,989,823.

The term "molecular beacons," as used herein, refers to a molecule or group of molecules (i.e., a nucleic acid molecule hybridized to an energy transfer complex or chromophore(s)) that can become detectable and can be attached to a biodetector/biosensor under preselected conditions. For example, an embodiment of the present invention includes an aptamer-bound fluorescence beacon that (a) quenches when a target analyte/biomarker is reversibly bound to the aptamer and (b) is detectable with a photodetector to quantify the concentration of target analyte/biomarker present.

As used herein, "analytes" and "biomarkers" are used interchangeably (i.e., "analyte/biomarker") to refer to naturally occurring and/or synthetic compounds, which are a marker of a condition (i.e., drug abuse), disease state (i.e., infectious diseases), disorder (i.e., neurological disorders), or a normal or pathologic process that occurs in a patient (i.e., drug metabolism). The term "analyte" or "biomarker," as used herein, can refer to any substance, including chemical and/or biological agents that can be measured in an analytical procedure.

Analytes/biomarkers that can be detected using the present invention include, but are not limited to, the following metabolites or compounds commonly found in bodily fluids: acetaldehyde (source: ethanol; diagnosis: intoxication), acetone (source: acetoacetate; diagnosis: diet or ketogenic/diabetes), ammonia (source: deamination of amino acids; diagnosis: uremia and liver disease), CO (carbon monoxide) (source: CH_2Cl_2, elevated % COHb; diagnosis: indoor air pollution); chloroform (source: halogenated compounds), dichlorobenzene (source: halogenated compounds), diethylamine (source: choline; diagnosis: intestinal bacterial overgrowth); H (hydrogen) (source: intestines; diagnosis: lactose intolerance), isoprene (source: fatty acid; diagnosis; metabolic stress), methanethiol (source: methionine; diagnosis: intestinal bacterial overgrowth), methylethylketone (source: fatty acid; diagnosis: indoor air pollution/diet), O-toluidine (source: carcinoma metabolite; diagnosis: bronchogenic carcinoma), pentane sulfides and sulfides (source: lipid peroxidation; diagnosis: myocardial infarction), H_2S (source: metabolism; diagnosis: periodontal disease/ovulation), MeS (source: metabolism; diagnosis: cirrhosis), Me_2S (source: infection; diagnosis trench mouth), αII-spectrin breakdown products and/or isoprostanes (source: cerebral spinal fluid, blood; diagnosis: traumatic or other brain injuries); prostate specific antigen (source: prostate cells; diagnosis: prostate cancer); and GLXA (source: glycolipid in Chlamydia; diagnosis: Chlamydia).

Additional analytes/biomarkers that can be detected using the present invention include, but are not limited to, illicit, illegal, and/or controlled substances including drugs of abuse (i.e., amphetamines, analgesics, barbiturates, club drugs, cocaine, crack cocaine, depressants, designer drugs, ecstasy, Gamma Hydroxy Butyrate—GHB, hallucinogens, heroin/morphine, inhalants, ketamine, lysergic acid diethylamide—LSD, marijuana, methamphetamines, opiates/narcotics, phencyclidine—PCP, prescription drugs, psychedelics, Rohypnol, steroids, and stimulants); allergens (i.e., pollen, spores, dander, peanuts, eggs, and shellfish); toxins (i.e., mercury, lead, other heavy metals, and *Clostridium Difficile* toxin); carcinogens (i.e., acetaldehyde, beryllium compounds, chromium, dichlroodiphenyltrichloroethane (DDT), estrogens, N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), and radon); and infectious agents (i.e., *Bordettella bronchiseptica,* citrobacter, *Escherichi coli,* hepatits viruses, herpes, immunodeficiency viruses, influenza virus, *listeria,* micrococcus, mycobacterium, rabies virus, rhinovirus, rubella virus, *Salmonella,* and yellow fever virus).

The term "bodily fluid," as used herein, refers to a mixture of molecules obtained from a patient. Bodily fluids include, but are not limited to, exhaled breath, whole blood, blood plasma, urine, semen, saliva, lymph fluid, meningal fluid, amniotic fluid, glandular fluid, sputum, feces, sweat, mucous, and cerebrospinal fluid. Bodily fluid also includes experimentally separated fractions of all of the preceding solutions or mixtures containing homogenized solid material, such as feces, tissues, and biopsy samples.

The term "biodetector/biosensor," as used herein, refers to the use of naturally-occurring and/or synthetic compounds as highly specific and sensitive detectors of various types of analytes/biomarkers. Naturally-occurring compounds such as antibodies, proteins, receptor ligands, and receptor proteins have been used to provide molecular recognition for a wide variety of target molecules in diagnostic assays. Alternatively, synthetic compounds such as aptamers have been manufactured that mimic naturally occurring mechanisms of DNA, RNA, and protein synthesis in cells to facilitate detection of target analytes/biomarkers.

The term "ex vivo," as used herein, refers to an environment outside of a patient. Accordingly, a sample of exhaled breadth collected from a patient is an ex vivo sample of bodily fluid as contemplated by the subject invention.

The term "surrogate marker," as used herein, refers to a molecule or compound that is detectable by means of its physical or chemical properties. As such, surrogate markers are detectable by a number of sensor technologies known in the art including, but not limited to, flow cytometers, semiconductive gas sensors; mass spectrometers; infrared (IR),

ultraviolet (UV), visible, or fluorescence spectrophotometers; gas chromatography, conductive polymer gas sensor technology; surface acoustic wave gas sensor technology; immunoassay technology, and amplifying fluorescent polymer (AFP) sensor technology.

A "patient," as used herein, describes an organism, including mammals, from which bodily fluid samples are collected in accordance with the present invention. Mammalian species that benefit from the disclosed systems and methods of diagnosis include, and are not limited to, apes, chimpanzees, orangutans, humans, monkeys; and domesticated animals (e.g., pets) such as dogs, cats, mice, rats, guinea pigs, and hamsters.

Nanoparticles

Nanostructure-based assemblies offer timely, and effective detection and notification of a condition, disorder, or disease in a patient via ex vivo analysis. Such assemblies are based on nanoparticles, which provide a mechanism for the detection of a target analyte/biomarker in a bodily fluid sample as well as a mechanism for the release of detectable surrogate markers to signal the presence of a target analyte/biomarker.

According to the present invention, nanoparticles can be produced in a wide range of sizes and shapes, and composed of a wide range of materials, or combination of materials, optimized for ex-vivo analysis. Contemplated shapes include, but are not limited to, spherical, elliptical, cubic, cylindrical, tetrahedron, polyhedral, irregular-prismatic icosahedral, and cubo-octahedral forms. Nanoparticles intended for ex-vivo use are of any dimension, preferably with a maximum dimension less than 500 nm, so as to ensure proper distribution at the cellular level. The "maximum dimension" of a nanoparticle is the maximum distance between any two points in the nanoparticle. In a preferred embodiment, the nanoparticles are in the form of tubular bodies (also known as "nanotubes"), which are either hollow or solid and include either open ends or one or both closed ends.

Methods of preparation of nanoparticles are well known in the art. For example, the preparation of monodisperse sol-gel silica nanospheres using the well-known Stober process is described in Vacassy, R. et al., "Synthesis of Microporous Silica Spheres," *J. Colloids and Interface Science,* 227, 302 (2000).

Nanoparticles, in accordance with the present invention, can be prepared from a single material or a combination of materials. For example, nanotubes can be prepared from either one or a combination of materials including, but not limited to, polymers, semiconductors, carbons, or Li^+ intercalation materials. Metal nanoparticles include those made from gold or silver. Semi-conductor nanoparticles include those made from silicon or germanium. Polymer nanoparticles include those made from biocompatible or biodegradable polymers. The ability to make nanoparticles from a wide variety of materials or combination of materials allows the creation of nanoparticles with desired biochemical properties such as biocompatibility, including immunogenic compatibility, and/or, biodegradability.

Nanoparticles of the present invention can be synthesized using a template synthesis method. For example, nanoparticles can be synthesized using templates prepared from glass (Tonucci, R. J. et al., *Science* 258, 783 (1992)), xeolite (Beck, J. S. et al., *J. Am. Chem. Soc.,* 114, 10834 (1992)), and a variety of other materials (Ozin, G. A., *Adv. Mater.,* 4, 612 1992)). Alternatively, nanoparticles can be prepared using a self-assembly process, as described in Wang, Z. L., "Structural Analysis of Self-Assembling Nanocrystal Superlattices," *Adv. Mater.,* 10(1):13–30 (1998).

In one embodiment, a nanostructure-based assembly of the invention contains a nanoparticle, which has one or more surfaces functionalized to allow attachment of biodetectors/biosensors to the surface. Such "functionalized" nanoparticles have at least one surface modified to allow for the directional delivery and/or controlled release of the payload and surrogate marker. In certain embodiments, the nanoparticle is formed with an interior void. Thus, different chemical and/or biochemical functional groups can be applied to the inside and/or outside surfaces of the nanoparticle. Alternatively, one chemical/biochemical species can be applied to the inside surface of the nanoparticle and a second species to the outside surface.

In another embodiment, the nanostructure-based assembly contains a nanoparticle formed with an interior void to contain a surrogate marker and a detechable end-cap with a detecting means attached thereto. In the presence of a target analyte/biomarker, the detecting means mechanically detaches the end-cap from the nanoparticle to release the surrogate marker for analysis by sensor technology.

In a preferred embodiment, the nanoparticle is in the form of a nanotube that is hollow and has a first open end and a second closed end. A surrogate marker is enclosed within the hollow interior of the nanotube. The first open end is blocked with an aptamer-end-cap that prevents the release of the surrogate marker located within the hollow interior of the nanotube.

Upon detecting a target analyte/biomarker by the aptamer attached to an end-cap, the surrogate marker is released with the uncapping of the nanoparticle. The uncapping mechanism may require the use of energy-bearing biomolecular motors such as, but not limited to, the actin-based system (Dickinson, R. B. and D. L. Purich, "Clamped filament elongation model for actin-based motors," *Biophys. J.,* 82:605–617 (2002)). Once the nanoparticle is uncapped, the released surrogate marker can then be detected using sensor technology known in the art including, but not limited to, gas chromatography, electronic noses, spectrophotometers to detect the surrogate marker's infrared (IF), ultraviolet (UV), or visible absorbance or fluorescence, or mass spectrometers.

Nanotubes

Depending upon the application, various types of sensors, for example, aptamers, antibodies/proteins, peptides, or high affinity ligands, can be linked to the uncapping/discharge mechanism of the nanocap-nanostructure-based assemblies of the invention. Thus, the uncapping mechanism can be linked to detection by the sensors on the nanocap-nanotube structure of surface markers on cells types (e.g., cancer cells), proteins in the blood (e.g., PSA for prostate cancer) or drugs in the body (e.g., illicit drugs or therapeutic drugs). These may or may not require the use of energy-bearing biomolecular motors such as, but not limited to, the actin-based system (Dickinson R. B. and Purich D. L., Biophys. . 2002 82:605–617).

In another embodiment, the nanocap (or "end-cap") is attached by electrostatic attraction between the nanocap and the nanotube. The cap is released in response to a change in the ionic strength of the medium according the nanotube. Alternatively, the cap can be held on by hydrogen bonding or by acid and/or basic sites on the nanocap/nanotube. The cap is released by a change in the pH or the surrounding

medium. The cap may also be held on by covalent bonds that can be cleaved by a specific enzyme, for example, a hydrolase enzyme.

The sensors can be designed to initiate release of payload contents (such as a surrogate marker) upon detecting stimuli. Such stimuli can include physical stimuli, for example, the temperature, pressure, velocity or acceleration of the nanoparticle; biological stimuli, for example, the presence of normal or abnormal cell types, cellular surface antigens, proteins, oligonucleotides, or toxins; or chemical stimuli, for example, pH, ionic strength, hydration state, redox state, or the presence of therapeutic agents, or toxic drugs such as nerve agents.

For example, one can achieve safe and effective intracellular surrogate marker release by attaching the nanocap to the nanotube with covalent bonds (e.g., S—H bonds) that are broken when a specific chemical signal (e.g., high reducing atmosphere of the cytoplasmic environment of the interior of a mammalian cell) is encountered. The ability to incorporate different types of sensor mechanisms for removal of the cap is an extremely powerful approach to the delivery and release (or uptake) of payload contents in an event- and site-specific manner. Specifically, by linking the uncapping mechanism to various sensing modes, the nanotubes based surrogate marker transport systems can be used to diagnose, treat, and monitor health status. For example, smart nanotubes can detect the appearance of cancer antigens on the walls of cancer cells, cause uncapping which in turn releases an indicator, which in turn makes the urine a distinct color or releases a nontoxic marker which can be readily detected in the breath, and thereby notifies the patient or his/her physician that a cancer cell(s) was encountered in his/her body.

Nanotube technology provides a method for delivering surrogate markers. In one embodiment, this is achieved using nanocaps that are firmly bound to the nanotube when the assembly is outside of the cell but are released, thus opening the nanotube and making the surrogate marker available, when the assembly is partitioned into the cell. For example, this can be accomplished using disulfide chemistry to couple the nanoparticle cap to the nanotube. The disulfide link between the nanotube and its nanocap is ideal because all living cells maintain a reducing environment within their cytoplasm. This contrasts with the oxidizing environment found outside the cell. The tripeptide glutathione (-gluatamyl-cysteinyl-glycine) plays a key role in this process. In its reduced form, glutathione possesses a free sulfhydryl capable of reducing disulfide bonds, forming a disulfide-linked glutathione dimer in the process. This species, in turn, is reduced by nicotinamide-dependent enzymes.

A number of patents and publications describe nanotube technology. For example, U.S. Pat. No. 5,482,601 to Ohshima et al. describes a method for producing carbon nanotubes. Other methods for making and using nanotubes include the non-carbon nanotubes of Zettl et al., U.S. Pat. No. 6,063,243, and the functionalized nanotubes of Fisher et al., U.S. Pat. No. 6,203,814.

For nanotubes, synthesis occurs within the membrane pores of a microporous membrane or other solid, as described in Charles R. Martin, "Nanomaterials: A Membrane-Based Synthetic Approach," *Science*, 266:1961–1966 (1994), using electrochemical or chemical methods. Depending on the membrane and synthetic method used, the nanotubes may be solid or hollow. Template membrane pore diameters can be varied to produce nanotubes having diameters as small as 5 nm to as large as 100 μm. Likewise, the template membrane thickness can be varied to given nanotubes having a length from as small as 5 nm to as large as 100 μm. Preferably, when the nanotube is intended for in vivo use, the nanotube is of length less than 500 μm and diameter less than 200 nm. Especially preferred nanotubes for ex vivo use have a maximum dimension less than 100 nm.

"Track-etch" polymeric or porous alumina membranes can be used in the preparation of nanotubes. Track-etch membranes prepared from polycarbonate and polyester are available from suppliers such as Osmonics (Minnetonka, Minn.) and Whatman (Maidstone, Kent UK). Tracl-etch membranes contain randomly distributed cylindrical pores of uniform diameter than run through the entire thickness of the membrane. Pore diameters as small as 10 nm are commercially available at pore densities of up to 10^9 pores per square centimeter.

Porous alumina membranes, which are commercially available from Whatman (Maidstone, Kent UK), are prepared electronically from aluminum metal. Pore diameters as small as 5 nm can be achieved at pore densities as high as 10^{11} pores per square centimeter. Membranes can be prepared having the membrane thickness from as small as 100 nm to as large as 100 μm.

Nanotubes can be synthesized such that both ends of the nanotube are open. Alternatively, nanotubes having one open end can be synthesized. Solid nanotubes can also be synthesized. Nanotubes with one closed end can be produced by template synthesis. Before the alumina template membrane is removed from the substrate aluminum surface, the pores in the alumina terminate into a non-porous alumina barrier layer (Hornyak, G. L., et al., "Fabrication, Characterization and Optical Properties of Gold-Nanoparticle/Porous-Alumina Composites: The Non-Scattering Maxwell-Garnett Limit," *J. Phys. Chem. B.*, 101:1548–1555 (1997)). This non-porous barrier layer is removed when the alumina membrane is stripped off the aluminum surface. However, if the template synthesis is completed before removal of the alumina from the aluminum, the bottoms of the nanotubes are closed. Dissolution of the alumina then liberates the nanotubes that are closed at one end and open at the other end.

Suitable end-caps used to block a nanotube opening include, for example, nanoparticles having a diameter slightly larger than the inside diameter of the nanotubes so as to occlude the open end of the nanotube. End-caps are any piece of matter and can be composed of materials that are chemically or physically similar (or dissimilar) to the nanotube. The end-cap can be a particle that has a maximum dimension of less than 100 μm. In a preferred embodiment, the end-cap is of a spherical or spheroidal form. However, end-caps of other shapes, including ellipsoidal, cylindrical, and irregular, can also be used.

A suitable end-cap can be attached to a nanotube by covalent bonds. For example, silica nanotubes and particles can be linked by disulphide bonds. Initially, the surface at the ends of silica nanotubes is functionalized with a —SH linker. This can be performed while the nanotubes are still embedded in the pores of the template membrane. This allows activation of the end surface without changing the chemical properties of the outer surface of the nanotubes.

If necessary, the inner surfaces of the nanotubes are protected with, for example, a silane group such as $(Me—O)_3$-$(CH_2)_3$—OH. After the protection step, the silica surface layers at the nanotube mouths are removed to expose fresh silica. The freshly-exposed silica will be reacted with the silane, such as $(Me—O)_3$—Si—$(CH_2)_3$—SH to attach the requisite —SH linker to the mouths of the nanotubes.

The length of the alkyl chain in this silane can be varied to allow placement of the —SH linker any desired distance from the nanotube mouth. Three —SH functionalities are then reacted with pyridine disulfide in order to obtain nanotubes with an activated disulfide bond at the nanotube ends.

The surface of the end-cap is then functionalized with the same —SH containing silane used on the mouths of the nanotubes. Hence, nanotubes with an activated disulfide at their mouths and end-caps with an —SH group on their surface are available for linkage through disulfide bond formation.

Other types of covalent bonds, for example amide and ester bonds, can be used to attach an end-cap to the nanotube. Siloxane based linking can also be used. This would be particularly useful when the cap is composed of silica as the silanol sites on the silica surface reacts spontaneously with siloxanes to form a covalent oxygen-silicon bond. For metal based nanotubes or end-caps, thiol linkers can be used for attachment. For example, molecule $(Me—O)_3—Si—(CH_2)_3—SH$ could be attached to a silica nanotube and a gold nanoparticle attached as the end-cap using the —SH end of this molecule. It is well known that such thiols form spontaneous As—S bonds with gold surfaces.

Contemplated end-caps for the invention include nanoparticles that can be electrophoretically placed within the mouths of nanotubes so that the entire mouth of the nanotube is blocked when disulfide bonds are formed between the nanotube and the nanoparticle as described in Miller, S. A. and C. R. Martin, "Electroosmotic Flow in Carbon Nanotube Membranes," *J. Am. Chem. Soc.*, 123(49):12335–12342 (2001).

The end-cap ("or nanocap") can be used to impart several novel functions and degrees of intelligence to the nanotube-nanocap delivery system. These include the sealing of the payload contents (such as the surrogate marker) within the nanotube in a cost-effective manner.

The nanocap can also provide a mechanism whereby the nanotube payload contents can be selectively released. For example, when used for the in-vivo delivery of a surrogate marker, the nanotube can be designed to release its payload either at the surface of the target cell or within its cytoplasm. This may be achieved by sensing a chemical, physical or biological signal present in the local environment. Alternatively, a remote external energy source, such as ultrasonic irradiation, can be used to selectively release the payload from the nanotube. Time-controlled degradation of the biomaterials used to construct the nanotube and/or nonocaps can also provide a release mechanism.

For example, a nanotube containing membrane is mounted in a U-tube cell with Platinum electrodes immersed into the buffer solution on either side of the membrane. The —SH-functionalized end-caps are added to the cathode half-cell. The buffer solution is maintained at pH=7 so that a small fraction of the —SH groups on the end-caps are deprotonated. These negatively charged particles are driven into the mouths of the nanotubes electrophoretically by using the Platinum electrodes to pass a constant current through the membrane. Hence, the electrophoretic force causes the end-caps to nestle into the nanotube mouths, where disulfide bond formation will occur.

As an alternative to the electrophoretic assembly method, —SH labeled end-caps can be suspended in solution together with the activated disulfide labeled nanotubes. Here, the nanoparticle caps can spontaneously self-assemble to the nanotubes. The self-assembly of gold nanospheres and latex particles to template prepared polymeric and metal nanowires is described by Sapp, S. A. et al., "Using Template-Synthesized Micro- and Nanowires as Building Blocks for Self-Assembly of Supramolecular Architectures," *Chem. Mater.*, 11:1183–1185 (1999).

In addition to —SH linking, other covalent linking methods can be used to link nanotubes and end-caps. Non-covalent linking methods can be used. These include, for example, DNA hybridization (Mirkin, C. A., "Programming the Self-Assembly of Two and Three-Dimensional Architectures with DNA and Nanoscale Inorganic Building Blocks," *Inorg. Chem.*, 39:2258–2272 (2000)), the biotin/avidin interaction (Connolly, S. and D. Fitzmaurice, "Programmed Assembly of Gold Nanocrystals in Aqueous Solution," *Adv. Mater.*, 11:1202–1205 (1999)), and antigen/antibody interactions (Shenton, W. et al., "Directed Self-Assembly of Nanoparticles into Macroscopic Materials Using Antibody-Antigen Recognition," *Adv. Mater.*, 11:449 (1999)).

Preferred nanotubes are those comprising silica or polymers. Silica nanotubes can be prepared using sol-gel template synthesis, as described in Lakshmi, B. B. et al., "Sol-Gel Template Synthesis of Semiconductor Oxide Micro- and Nanostructures," *Chem. Mater.*, 9:2544–2550 (1997); Lakshmi, B. B. et al., "Sol-Gel Template Synthesis of Semiconductor Nanostructures," *Chem. Mater.*, 9:857–862 (1997). The template membrane is immersed into a standard tetraethylorthosilicate sol so that the sol fills the pores. After the desired emersion time, the membrane is removed, dried in air, and then cured at 150° C. This yields silica nanotubes lining the pore walls of the membrane plus silica surface films on both faces of the membrane. The surface films are removed by briefly polishing with slurry of alumina particles. The nanotubes are then liberated by dissolving the template membrane and collected by filtration.

The outside diameter of the nanotube can be controlled by varying the pore diameter of the template membrane, the length of the nanotube can be controlled by varying the thickness of the template membranes, and the inside diameter of the nanotube can be controlled by varying the immersion time in the sol.

Polymer nanotubes can be prepared from many substances that are composed of monomer units. "Monomer units," as used herein, refers to the individual moieties that are repeated to form "polymers." Multiple monomer units are covalently attached when tin the form of a backbone of a polymer. Polymers that are made from at least two different types of monomer units are referred to as "copolymers." Polymerizing or copolymerizing describes the process by which multiple monomers are reacted to form covalently linked monomer units that form polymers or copolymers, respectively. A discussion of polymers, monomer units, and the monomers from which they are made may be found in Stevens, *Polymer Chemistry: An Invasion;* 3rd ed., Oxford University Press (1999).

Polymeric nanotubes can be prepared using a solution deposition method as described in Depak, V. M. and C. R. Martin, "Preparation of Polymeric Micro- and Nanostructures Using a Template-Based Deposition Method," *Chem. Mater.*, 11:1363–1367 (1999). This method entails depositing a solution of the desired polymer within the pores of the template membrane and allowing the solvent to evaporate. In addition, polymer nanotubes can be prepared by polymerizing a monomer of a monomer within the pore as described by Martin, C. R., "Template Synthesis of Electronically Conductive Polymer Nanostructures," *Acc. Chem. Res.*, 28:61–68 (1995).

Preferred polymers include polystyrene, polyorganosiloxane, poly(methyl methacrylate), polystyrene, polylactic acids, and other biodegradable polymers, acrylic latexes, polyorganosilioxane, cellulose polyethylene, poly(vinyl chloride), poly(ethyl methacrylate), poly(tetrafluoroethylene), poly(4-iodostyrene/divinyl/benzene), poly(4-vinylpyridine/vidinylbenzene), poly(styrene/divinyl benzene), crosslinked melamine particles, phenolic polymer colloids, polyamide 6/6, natural rubber, naturally occurring biopolymers such a salgenates, and collagen, or mixtures thereof.

Functionalization of Nanoparticle Surface

Methods used to functionalize a nanoparticle surface, in accordance with the present invention, depend on the composition of the nanoparticle and are well known in the art. For example, functionalization of silica nanoparticles is accomplished using silane chemistry. The detecting means and/or the surrogate marker can be attached to the surfaces of the nanoparticle by attaching them to the surface of the nanoparticle while the nanoparticle is still embedded with a template. Alternatively, while the nanoparticle is embedded in the templates, a hydrolytically unstable silane is reacted with surface silanol sites on nanoparticle to obtain covalent oxygen/silicon bonds between the surface and the silane. Either the detecting means and/or the surrogate marker can then be attached to the surface of the nanoparticle after dissolution of the template.

The surface of polymer-based nanoparticles can also be functionalized using well-known chemical methods. For example, the methods employed for polylactide synthesis allow for differential end-functionalization. Polymerization occurs by an insertion mechanism mediated by Lewis acids such as Sn^{2+} whose bonds with oxygen have significant covalent character. An alcohol complexed with the metal ion initiates polymerization, which continues by stepswise ring-opening of the lactide monomers to generate a new alkoxide-metal complex capable of chain growth. The polymer molecular weight can be controlled by the molar ratio of initiating alcohol to the lactide monomer. The resulting polyester possesses directly with a hydroxyl terminus (from the first monomer) and either a detecting means and/or surrogate marker at the ester terminus determined by the structure of the initiating alcohol. The latter can contain a variety of detecting means and/or surrogate markers.

Additionally, the detecting means and/or surrogate marker can be introduced by copolymerization. Naturally amino acids re sterically similar to lactic acid but offer a variety of functional groups on their side-chains (—OH, —CO_2H, —NH_2, —SH, etc.) Monomer derived from an amino acid and lactic acid can be synthesized by standard methods and used for random copolymerization with lactide.

By functionalizing the nanoparticle with either a detecting means and/or surrogate marker, the present invention provides nanostructure-based assemblies that can immobolize a specific protein or cell. See Langer, R., "Tissue Engineering," *Mol. Ther.*, 1:12–15 (2000). Detecting means, for example proteins, including antibodies or peptides, RNA, or DNA, aptamers, cellular receptor ligands, are attached to the nanoparticle surface. Such detecting means may be attached covalently, including attachment via functional groups introduced by the functionalization of the surface. Alternatively, the detecting means may be covalently attached via linker molecules. Non-covalent linkages can also be used to attach detecting means and/or surrogate markers to the nanoparticle. Examples of non-covalent linkages include, and are not limited, absorption via hydrophobic binding or Van der Waals forces, hydrogen bonding, acid/base interactions, and electrostatic forces.

For nanoparticles comprising a hollow void in which the surrogate marker can be contained, a surrogate marker can be loaded into the void using an electrophoretic force. (See Miller, S. A. and C. R. Martin, "Electroosmotic Flow in Carbon Nanotube Membranes," *J. Am. Chem. Soc.*, 123(49): 12335–12342 (2001)). Alternatively, nanoparticles embedded within the synthesis membrane can be filled with a surrogate marker by vacuum filtering a solution containing the surrogate marker through the synthesis membrane. (See Parthasarathy, R. and C. R. Martin, *Nature*, 369:298 (1994)). For nanoparticles prepared by formation within an alumina template film prior to removal of the alumina from the underlying aluminum surface, they can be filled by simply applying a solution containing the surrogate marker to the surface of the film (where the opening to the hollow void is located) and allowing the solvent to evaporate. Multiple applications can be used, if needed.

Means for Detecting Target Analytes/Biomarkers

The present invention contemplates using known bifunctional or hybrid molecules for detecting target analytes/biomakers. Some of these molecules include, but are not limited to, chimeric antibodies; bispecific antibodies (i.e., antibodies produced through enzymatic digestion of parent antibodies and controlled reconstitution using Fab fragments obtained from two different parents); conventional immunoconjugates, which can include an imaging agent covalently attached or chelated to an antibody or antibody fragment through established immunochemical methods; and fusion proteins, generated from hybrid genes developed and expressed through recombinant methods.

According to the present invention, other contemplated means for detecting a target analyte/biomarker include antibodies, antigens, haptens and nucleic acid probes with site-directed effectors (i.e., fluorophores). DNA, including branched DNA, can also be used in accordance with the present invention as a means for detecting target analytes/biomarkers. For example, it has been shown that particular proteins recognize and bind to specific sites on the DNA. See Seeman, *Clin. Chem.*, 39:722 (1993).

The present invention preferably utilizes aptamers to non-invasively detect drugs, biomarkers, and other analytes in exhaled breath and other bodily fluids, such as blood. In a preferred embodiment, the invention includes aptamers in combination with nanotechnology (i.e., nanotubes) to provide an effective method for signaling the presence of a target analyte/biomarker in bodily fluids, particularly in blood.

The discover of the SELEX™ (Systematic Evolution of Ligands of EXponential enrichment) methodology enabled the identification of aptamers that recognize molecules other than nucleic acids with high affinity and specificity (Ellington and Szostak, "In vitro selection of RNA molecules that bind specific ligands," *Nature*, 346:818–822 (1990); Gold et al., "Discovery of oligonucleotide functions," *Ann. Rev. Biochem.*, 64:763–797 (1995); Tuerk and Gold, "Systematic evolution of ligands by exponential enrichment—RNA ligands to bacteriophage-T4 DNA-polymerase," *Science*, 249:505–510 (1990)). Aptamers have been selected to recognize a broad range of targets, including small organic molecules as well as large proteins (Gold et al., supra.; Osborne and Ellington, "Nucleic acid selection an the challenge of combinatorial chemistry," *Chem. Rev.*, 97:349–370 (1997)).

The aptamers derived from the SELEX methodology may be utilized in the present invention. The SELEX methodology enables the production of aptamers, each of which have a unique sequence and the property of binding specifically to a desired target compound or molecule. The SELEX methodology is based on the insight that nucleic acids have sufficient capacity for forming a variety of two- and three-dimensional structures and sufficient chemical versatility available within their monomers to act as ligands (form specific binding pairs) with virtually any chemical compound, whether monomeric or polymeric. Molecules of any size or composition can serve as targets. See also Jayasena, S., "Aptamers: An Engineering Class of Molecules That Rival Antibodies for Diagnostics," *Clinical Chemistry,* 45:9, 1628–1650 (1999).

Aptamers that can be used in the present invention include those described in U.S. Pat. No. 5,656,739 (hereinafter the '739 patent), which discloses the advantages of synthetic oligonucleotides as assembly templates. The '739 patent describes nucleic acids as particularly useful assembly templates because they can be selected to specifically bind nonoligonucleotide target molecules with high affinity (e.g.; Tuerk and Gold (1990), supra), and because they can hybridize by complementary base pairing. Both forms of recognition can be programmably synthesized in a single molecule or hybridized into a single discrete structure.

As described herein, nanoparticles can be prepared with hollow interiors and include functionalized end-caps with detecting means. A variety of methods are available to functionalize an end-cap, depending on the composition of the end-cap. For example, an end-cap can be functionalized using well-known chemical methods such as those employed for polylactide synthesis. Detecting means (i.e., aptamers, proteins, antibodies) can be introduced to "functionalized" end-caps by copolymerization. For example, monomers derived from an amino acid or lactic acid can be synthesized using standard methods and then used for random copolymerization with lactic. Such functionalized end-caps can allow for the application of the detecting means to the end-cap.

In one embodiment, the detecting means are aptamers that can be attached to proteins utilizing methods well known in the art (see Brody, E. N. and L. Gold, "Aptamers as therapeutics and diagnostics agents," *J. Biotechnol.* 74(1): 5–13 (2000) and Brody, E. N. et al., "The use of aptamers in large arrays for molecular diagnostics," *Mol Diagn.,* 4(4):381–8 (1999)). For example, photo-cross-linkable aptamers allow for the covalent attachment of aptamers to proteins. Such aptamer-linked proteins can then be immobilized on a functionalized end-cap of a nanoparticle. For example, aptamer-linked proteins can be attached covalently to a nanoparticle end-cap, including attachment of the aptamer-linked protein by functionalization of the end-cap surface. Alternatively, aptamer-linked proteins can be covalently attached to an end-cap via linker molecules. Non-covalent linkage provides another method for introducing aptamer-linked proteins to an end-cap. For example, an aptamer-linked protein may be attached to an end-cap by absorption via hydrophilic binding or Van der Waals forces, hydrogen bonding, acid/base interactions, and electrostatic forces.

Aptamer-end-caps, according to the present invention, are bound to the nanoparticle until the detection of a target analyte/biomarker by the aptamer. End-caps are attached to nanoparticles using a variety of methods. Methods for attaching an end-cap to a nanoparticle include, but are not limited to, using: electrostatic attraction, hydrogen bonding, acid and/or basic sites located on the end-cap/nanoparticle, covalent bonds, and other chemical linkages.

Sensor Technology

Sensor technology is used by the present invention to detect the presence of a surrogate marker released from a nanoparticle-based sensor in a bodily fluid sample. The surrogate marker signifies the presence and quantity of a target analyte/biomarker. The present invention contemplates using sensor technology based on surface acoustic wave (SAW) sensors. These sensors oscillate at high frequencies and respond to perturbations proportional to the mass load of certain molecules. This occurs in the vapor phase on the sensor surface. The resulting frequency shift is detected and measured by a computer. Usually, an array of sensors (4–6) is used, each coated with a different chemoselective polymer that selectively binds and/or absorbs vapors of specific class of molecules. The resulting array, or "signature" identifies specific compounds. Sensitivity of the arrays is dependent upon the homogeneity and thickness of the polymer coating.

Surface-acoustic wave (SAW) gas-sensors generally include a substrate with piezoelectric characteristics covered by a polymer coating, which is able to selectively absorb the target surrogate markers. The variation of the resulting mass leads to a variation of its resonance frequency. This type of sensor provides very good mass-volume measures of the surrogate markers. In the SAW devices, the surrogate marker is used to propagate a surface acoustic wave between sets of interdigitated electrodes. The chemoselective material is coated on the surface of the transducer. When a surrogate marker interacts with the chemoselective material coated on the substrate, the interaction results in a change in the SAW properties, such as the amplitude or velocity of the propagated wave. The detectable change in the characteristics of the wave indicates the presence and concentration of the surrogate marker (and corresponding target analyte/biomarker).

Certain embodiments use known SAW devices described in numerous patents and publications, including U.S. Pat. Nos. 4,312,228 and 4,895,017, and Groves W. A. et al., "Analyzing organic vapors in exhaled breath using surface acoustic wave sensor array with preconcentration: Selection and characterization of the preconcentrator adsorbent," *Analytica Chimica Acta,* 371:131–143 (1988).

Other types of chemical sensors known in the art that use chemoselective coating applicable to the operation of the present invention include bulk acoustic wave (BAW) devices, plate acoustic wave devices, interdigitated microelectrode (IME) devices, optical waveguide (OW) devices, electrochemical sensors, and electrically conducting sensors.

In another embodiment, the invention uses fluid sensor technology, such as commercial devices known as "artificial noses," "electronic noses," or "electronic tongues." These devices are capable of qualitative and/or quantitative analysis of simple or complex gases, vapors, odors, liquids, or solutions. A number of patents and patent applications which describe fluid sensor technology include the following: U.S. Pat. Nos. 5,945,069; 5,918,257; 5,891,398; 5,830,412; 5,783,154; 5,756,879; 5,605,612; 5,252,292; 5,145,645; 5,071,770; 5,034,192; 4,938,928; and 4,992,244; and U.S. patent application Ser. No. 2001/0050288. Certain sensitive, commercial off-the-shelf electronic noses, such as those provided by Cyrano Sciences, Inc. ("CSI") (i.e., CSI's portable Electronic Nose and CSI's Nose-Chip™ integrated circuit for odor-sensing—U.S. Pat. No. 5,945,069), can be

used in the present invention to detect the presence of surrogate markers in bodily fluid samples.

Other embodiments of the present invention use sensor technology selected from semiconductive gas sensors; mass spectrometers; and IR, UV, visible, or fluorescence spectrophotometers. With these sensors, a surrogate marker changes the electrical properties of the semiconductors by making their electrical resistance vary, and the measurement of these alternatives allows the determination of the concentration of surrogate markers present in the sample. The methods and apparatus used for detecting surrogate markers generally have a brief detection time of a few seconds.

Additional recent sensor technologies included in the present invention include apparatus having conductive-polymer gas-sensors ("polymeric"), aptamer biosensors, and amplifying fluorescent polymer (AFP) sensors.

Conductive-polymer gas-sensors (also referred to as "chemoresistors") are coated with a film sensitive to the molecules of certain surrogate markers. On contact with the molecules, the electric resistance of the sensors change and the measurement of the variation of this resistance enable the concentration of the sensors surrogate substance (and corresponding target analyte/biomarker) to be determined. An advantage of this type of sensor is that it functions at temperatures close to ambient. Different sensitivities for detecting different surrogate markers can be obtained by modifying or choosing an alternate conductive polymer.

Polymeric gas sensors can be built into an array of sensors, where each sensor responds to different gases and augment the selectivity of the surrogate marker.

Aptamer biosensors can be utilized in the present invention for detecting the presence of surrogate markers in bodily fluid samples. Aptamer biosensors are resonant oscillating quartz sensors that can detect minute changes in resonance frequencies due to modulations of mass of the oscillating system, which results from a binding or dissociation event.

Similarly, amplifying fluorescent polymer (AFP) sensors may be utilized in the present invention for detecting the presence of surrogate markers in bodily fluid samples. AFP sensors are extremely sensitive and highly selective chemosensors that use amplifying fluorescent polymers. When vapors bind to thin films of the polymers, the fluorescence of the film decreases. A single molecule binding event quenches the fluorescence of many polymer repeat units, resulting in an amplification of the quenching. The binding of surrogate markers to the film is reversible, therefore the films can be reused.

The results from the sensor technology analysis of the bodily fluid samples are optionally provided to the user (or patient) via a reporting means. In one embodiment, the sensor technology includes the reporting means. Contemplated reporting means include a computer processor linked to the sensor technology in which electronic or printed results are provided. Alternatively, the reporting means can include a digital display panel, transportable read/write magnetic media such as computer disks and tapes which can be transported to and read on another machine, and printers such as thermal, laser or ink-jet printers for the production of a printed report. The reporting means can provide the results to the user (or patient) via facsimile, electronic mail, mail or courier service, or any other means of safely and securely sending the report to the patient. Interactive reporting means are also contemplated by the present invention, such as an interactive voice response system, interactive computer-based reporting system, interactive telephone touch-tone system, or other similar system. The report provided to the user (or patient) may take many forms, including a summary of analyses performed over a particular period of time or detailed information regarding a particular bodily fluid sample analysis. Results may also be used to populate a financial database for billing the patient, or for populating a laboratory database or a statistical database.

Specific conditions/diseases that can be detected using the present invention are listed in *Merck Manual Diagnosis and Therapy*, 17th *ed.*, Merck & Company, Inc., 1999, which include, but are not limited to, blood disorders (i.e., blood coagulation disorders, lymphedema, hemochromatosis, leukemia, lymphedema, myelodysplastic syndromes, neutropenia), cancers (i.e., brain tumors, breast cancer, colorectal cancer, lymphomas, lung cancer, prostate cancer), cardiovascular disorders (i.e., coronary artery disease, congenital heart disease, atherosclerosis, aneurysm, peripheral arterial disease), disorders of the esophagus (i.e., achalasia), Barrett's esophagus), intestinal disorders (i.e., Celiac disease, Crohn's disease, inflammatory bowel disease), liver diseases and disorders (i.e., cirrhosis of the liver, hepatitis, Wilson's disease), pancreatic diseases and disorders (i.e., pancreatitis, cystic fibrosis), disorders of the ear, nose or throat (i.e., Meniere's disease, strep throat), endocrine disorders (i.e., congenital adrenal hyperplasia, diabetes, hypoglycemia, hyperparathyroidism, hypoparathyroidism, and Cushing's syndrome), eye disorders (i.e., retinoblastoma, uveitis, Lebers optic neuropathy, keratoconus), genetic disorders (i.e., Marfan's syndrome, porphyries, Huntingdon's disease, normal pressure hydrocephalus (NPH), Wilson's disease), gynecologic disorders (i.e., polycystic ovarian syndrome, endometriosis), immune disorders (i.e., AIDS, Addison's disease, Lupus, Sjogren's syndrome), infectious diseases (i.e., bacterial (rickettsial diseases, anthrax, endocarditis, salmonellosis), viral (chickenpox, herpes, influenza, pneumonia, shingles, West Nile virus), fungal (aspergillosis), parasitic (malaria, scabies, pinworms), prion (Creutzfeldt Jakob Disease)), metabolism disorders (fatty oxidation disorders, glycogen storage disorders I and II, glutaric acidemia), musculkoskeletal disorders (osteoporosis), neurological disorders (Alzheimer's disease, meningitis, demyelinating diseases), respiratory conditions, and urological disorders (hemolytic uremic syndrome, urinary tract infections).

EXAMPLE 1

Diagnosis of Traumatic Brain Injury (TBI)

The present invention provides methods for diagnosing acute and/or chronic neurological diseases and disorders (i.e., Alzheimer's disease, Parkinson's disease) and other clinical conditions by detecting in vitro analytes/biomarkers of oxidative stress. For example, it is known that certain αII-spectrin breakdown products, including isoprostane, levels increase in cerebral spinal fluid and blood after traumatic brain injury.

In accordance with the present invention, nanostructure-based assemblies are created in which the detecting means is designed to specifically detect and localize the assembly to isoprostanes and/or αII-spectrin breakdown products. In a preferred embodiment, the detecting means is an aptamer designed to bind to isoprostanes and/or αII-spectrin breakdown products. A sample of a patient's bodily fluid (i.e., blood or cerebral spinal fluid) is placed into a sealed vial containing the nanostructure-based assemblies designed as described above.

In one embodiment, the sample is incubated at an elevated temperature to allow any surrogate markers that were released from the nanostructure-based assemblies to diffuse out of the liquid phase into the "headspace" (gas phase) within the sealed vial. Under constant conditions of temperature, pressure, and equilibration time, the vapor phase in the sample vial is sampled and separated on a suitable gas chromatographic column. The surrogate markers are detected using flame ionization detector or nitrogen phosphorous detector.

In another embodiment, an "electronic nose" is used to detect and measure the amount of surrogate marker released in the sample via to assess whether the patient suffers from traumatic brain injury. As contemplated by the subject invention, the electronic nose can include the following components: (a) a sensor having an array of polymers capable of detecting the presence of the surrogate marker in the headspace of the vial, wherein the sensor responds to the surrogate marker by changing the resistance in each polymer resulting in a pattern change in the sensor array; (b) a processor for receiving the change in resistance, comparing the change in resistance with a previously measured change in resistance, and identifying the presence of the surrogate marker from the pattern change, and (if requested) the concentration of the surrogate marker from the amplitude. In a related embodiment, the sensor can include measuring circuitry and an output device can be included (i.e., screen display, audible output, printer). The processor can include a neural network for comparing the change in resistance with a previously measured change in resistance to find a best match.

By measuring isoprostane level and/or αII-spectrin breakdown products using the nanostructure-based assemblies of the invention, a clinician can not only identify if a patient is suffering from TBI, but once diagnosed, a clinician can follow the course of the brain injury. Moreover, by continuously testing samples of bodily fluid in accordance with the present invention, it is possible to evaluate th the efficacy of interventions in real-time for treating TBI. Accordingly, the method of the present invention can also evaluate pharmacodynamics and pharmacokinetics for drug interventions in individuals.

EXAMPLE 2

Diagnosis of Bronchogenic Carcinoma

In an embodiment, a nanostructure-based assembly of the present invention can be designed to detect bronchogenic carcioma. Bronchogenic carbinomas produce carcinoma metabolites that cause the occurrence of O-toluidine in exhaled breath. The detecting means of the nanostructure-based assembly can be in the form of an aptamer. Using routine techniques, the aptamer can be designed so that it is specific for O-toluidine (O-toluidine-aptamer). The O-toluidine-aptamer can be linked to a nanoparticle using functionalization methods as described above. The nanoparticle contains a surrogate marker that would be released in the presence of O-toluidene. Upon exposing the nanostructure-based assembly to a sample of bodily fluid (i.e., exhaled breath) suspected of containing O-toluidine, the O-toluidine-aptamer specifically binds to any O-toluidine present in the sample and causes the release of the surrogate marker to generate a signal that O-toluidine is present in the bodily fluid sample.

In one embodiment, sensor technology used to detect the surrogate marker in the sample has the following components: (a) a surface-acoustic wave sensor capable of detecting the presence of the surrogate marker in the mixture of bodily fluid (i.e., expired breath) and the nanostructure-based assembly, wherein the sensor responds to the surrogate marker by a shift in the resonant frequency; (b) an oscillator circuit having the sensor as an active feedback element; (c) a frequency counter in communication with the oscillator circuit to measure oscillation frequency which corresponds to resonant frequency of the sensor; and (d) a processor for comparing the oscillation frequency with a previously measured oscillation frequency with a previously measured oscillation frequency of the surrogate marker and determining presence and concentration of the surrogate marker therefrom. The sensor technology of the present invention an include measuring circuitry and an output device (i.e., screen display, audible output, and printer).

The processor can include a neural network (not shown) for pattern recognition. Artificial Neural Networks (ANNs) are well understood by the skilled artisan. ANNS are self-learning; the more data presented, the more discriminating the instrument becomes. By running many standard samples of bodily fluids and storing the results in computer memory, the application of ANN enables the sensor technology to "understand" the significance of the sensor array outputs better and to use this information for future analysis, "Learning" is achieved by varying the emphasis, or weight, that is placed on the output of one sensor versus another. The learning process is based on the mathematical, or "Euclidean," distance between data sets. Large Euclidean distances represent significant differences in sample-to-sample surrogate marker characteristics.

Thus, a time- and cost-efficient test for the presence of bronchogenic carcinoma is provided.

EXAMPLE 3

Diagnosis of Prostate Cancer

In another embodiment, a detecting means is designed for a biomarker of a specific cancer, i.e., prostate cancer. Prostate cancers produce a protein, prostate specific antigen (PSA). In a preferred embodiment, the detecting means is an aptamer designed to specifically bind to PSA (PSA-aptamer). The PSA-aptamer can be attached to a nanoparticle using functionalization methods as described above. The nanoparticle also includes a surrogate marker that is released in the presence of PSA. In one embodiment, the PSA-aptamer and the surrogate marker are attached to the surface of the nanoparticles using functionalized methods as described above. The nanostructure-based assembly is introduced to a sample of bodily fluid (i.e., blood) to identify the presence of PSA. Where PSA is present in the bodily fluid sample, the PSA-aptamer will bind to PSA and affect the release of the surrogate marker form the nanoparticle to signal the presence and concentration of PSA in the bodily fluid sample.

In a preferred embodiment, the nanostructure-based assembly is composed of a hollow nanoparticle. The detecting means, i.e., PSA-apatamer, is attached to an end-cap that fits onto an opening of a nanoparticle. The nanoparticle preferably encapsulates a surrogate marker. IN a rapid test for the presence of prostate cancer, or a recurrence of prostate cancer, the PSA-nanostructure-based assembly is mixed with a sample of bodily fluid (i.e., exhaled breath, exhaled condensates with proteins). The surrogate marker is released from the nanoparticle after PSA (the biomarker of interest) interacts with the PSA-aptamer and "uncaps" the

nanoparticle. Using any of a number of previously disclosed sensor technologies, the surrogate marker is detected in the sample of exhaled breath to indicate the presence and/or concentration of PSA in the sample.

In a related embodiment, the sensor technology used to detect any surrogate markers in the sample of bodily fluids (i.e., exhaled breath or exhaled condensates with proteins) comprises at least one polymer (or an array of polymers) exposed to the sample-nanoparticle mixture. Thus, a variety of nanostructure-based assemblies can be applied to a sample of bodily fluid to detect more than one target analyte/biomarker. Each of the individual polymers of the sensor technology swells differently in the presence of a specific surrogate marker, creating a change in the resistance of the membrane and generating an analog voltage in response to the specific surrogate marker ("signature"). Based on the pattern change in the sensor array, the normalized change in resistance is then transmitted to a processor to identify the type and quantity of the surrogate marker. The unique response results in a distinct electrical fingerprint characterizing the substance. The pattern of resistance changes of the array indicates the presence of a specific surrogate marker and the amplitude of the pattern indicates its concentration.

In another embodiment, the sensor technology can be designed so that patients provide a sample of bodily fluid (i.e., exhaled breath or exhaled condensates with proteins) directly into the device. For example, a mouthpiece or nosepiece is provided for interfacing a patient with the sensor technology to readily transmit a sample of exhaled breath to the sensor technology to be mixed with nanostructure-based assemblies. This, however, is not a limitation on the invention as samples of bodily fluids can be sampled immediately or stored.

All patents, patent applications, provisional applications, and publications referred to or cited herein are incorporated by reference in their entirety, including all figures and tables, to the extent they are not inconsistent with the explicit teachings of this specification.

It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application.

We claim:

1. A method for detecting ex vivo a target analyte/biomarker comprising:

(a) collecting a sample of bodily fluid in a container, wherein the sealed container comprises the sample of bodily fluid and a headspace;

(b) mixing ex vivo to the bodily fluid in the container a nanostructure-based assembly that comprises a surrogate marker where the surrogate marker is released from the nanostructure-based assembly in the presence of a target analyte/biomarker;

(c) applying sensor technology to the headspace or bodily fluid sample in the container, which contains the mixture of nanostructure-based assembly and bodily fluid sample in the container, wherein the sensor technology detects a surrogate marker released from the nanostructure-based assembly in the sealed container; and

(d) determining whether the sample contains the target analyte/biomarker by using the sensor technology to detect the presence of the surrogate marker, wherein the detection of the surrogate marker indicates the presence of the target analyte/biomarker in the sample of bodily fluid in the container.

2. The method according to claim **1**, wherein the nanostructure-based assembly comprises at least one nanotube comprising a hollow interior, a first end, a second end, surrogate marker located within the hollow interior, and an end-cap, wherein the first end is open and the second end is closed, the first end being blocked with the end-cap to prevent the release of the surrogate marker, wherein a means for detecting the target analyte/biomarker is attached to the end-cap; wherein the means for detecting the target analyte/biomarker can bind to the target analyte/biomarker; and wherein when the means for detecting the target analyte/biomarker binds to the target analyte/biomarker, the end-cap is displaced from the first end to release the surrogate marker.

3. The method according to claim **2**, wherein the means for detecting to the target analyte/biomarker is selected from the group consisting of aptamers, antibodies, proteins, and receptor ligands.

4. The method according to claim **3**, wherein the aptamer is capable of binding to the target analyte/biomarker selected from the group consisting of αII-spectrin breakdown products and protease-specific spectrin breakdown products.

5. The method according to claim **1**, wherein the target analyte/biomarker is a nucleic acid, a protein, an illicit drug, an explosive, a toxin, a pharmaceutical, a carcinogen, a poison, an allergen, or an infectious agent.

6. The method according to claim **1**, wherein the target analyte/biomarker is selected from the group consisting of acetaldehyde, acetone, ammonia, CO, chloroform, dichlorobenzene, diethylamine, hydrogen, isoprene, methanethiol, methylethylketone, O-toluidine, pentane sulfides and sulfides, H_2S, MES, and Me_2S.

7. The method according to claim **1**, wherein the bodily fluid sample is selected from the group consisting of: exhaled breath, blood, urine, bile, sweat, feces, semen, saliva, mucus, and cerebral spinal fluid.

8. The method according to claim **1**, wherein the sensor technology is selected from the group consisting of surface-acoustic-wave sensors; fluid sensor technology; semiconductive gas sensors, mass spectrometers; IR, UV, visible and fluorescence spectrophotometers; conductive-polymer gas-sensors; aptamer biosensors; and amplifying fluorescent polymer sensors.

9. The method according to claim **1**, wherein the sensor technology comprises:

(a) a surface-acoustic wave (SAW) sensor capable of detecting the presence of a surrogate marker in a sample of bodily fluid, wherein the SAW sensor responds to the surrogate marker by a shift in the resonant frequency;

(b) an oscillation circuit having the SAW sensor as an active feedback element;

(c) a frequency counter in communication with said oscillator circuit to measure oscillation frequency which corresponds to resonant frequency of the SAW sensor; and

(d) a processor for comparing the oscillation frequency with a previously measured oscillation frequency of the surrogate marker and determining presence and concentration of the surrogate marker therefrom.

10. The method according to claim **1**, wherein the sensor technology comprises:

(a) a sensor having an array of polymers capable of detecting the presence of the surrogate marker in the sample of bodily fluid, wherein said sensor responds to

the surrogate marker by changing the resistance in each polymer resulting in a pattern change in the sensor array;

(b) a processor for receiving the change in resistance, comparing the change in resistance with a previously measured change in resistance, and identifying the presence of the surrogate marker from the pattern change and the concentration of the surrogate marker from the amplitude.

11. The method according to claim **1**, wherein the nanostructure-based assembly comprises at least one nanoparticle comprising a surrogate marker and a means for detecting a target analyte/biomarker, wherein the means for detecting the target analyte/biomarker is bound to the nanoparticle in such a way as to affect the release of the surrogate marker when in the presence of a target analyte/biomarker; wherein when the means for detecting the target analyte/biomarker is in the presence of the target analyte/biomarker, the surrogate marker is released for detection by the sensor technology.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 7,052,854 B2
APPLICATION NO. : 10/678506
DATED : May 30, 2006
INVENTOR(S) : Richard J. Melker et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Cover Page,
"(75) Inventors: **Richard J. Melker**, Gainesville, FL (US); **Ronald L. Hayes**, Gainesville, FL (US); **Ka-Wang Kevin Wang**, Gainesville, FL (US); **Donn Michael Dennis**, Gainesville, FL (US)" should read --(75) Inventors: **Richard J. Melker**, Gainesville, FL (US); **Ronald L. Hayes**, Gainesville, FL (US); **Ka-Wang Kevin Wang**, Gainesville, FL (US); **Donn Michael Dennis**, Gainesville, FL (US); **Charles R. Martin**, Gainesville, FL (US); **Jon D. Stewart**, Gainesville, FL (US)--

Cover Page,
Abstract, Line 9, "apparatus" should read --"aptamers"--.

Column 8,
Line 31, "dichlroodiphenyltrichloroethane" should read --dichlorodiphenyltrichloroethane--.

Column 10,
Line 60, "Biophys. .2002" should read --Biophys. J. 2002--.
Line 64, "medium according" should read --medium surrounding--.

Column 11,
Line 24, "nanotubes based" should read --nanotube based--.

Column 13,
Line 33, "(or nanocap")" should read --(or "nanocap")--.

Column 13,
Line 48, "nonocaps" should read --nanocaps--.

Column 14,
Line 54, "Polymer Chemistry: An Invasion" should read --Polymer Chemistry: An Invitation--.

Column 15,
Line 4, "polyorganosilioxane" should read --polyorganosiloxane--.
Line 11, "a salgenates" should read --as algenates--.
Line 23, "in the templates" should read --in the template--.

Column 16,
Line 58, "Discovery of" should read --Diversity of--.

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 7,052,854 B2
APPLICATION NO. : 10/678506
DATED : May 30, 2006
INVENTOR(S) : Richard J. Melker et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 20,
Line 40, "musculkoskeletal disorders" should read --musculoskeletal disorders--.

Column 21,
Line 13, "the sample via to" should read --the sample vial to--.
Line 50, "Bronchogenic carbinomas" should read --Bronchogenic carcinomas--.

Column 22,
Line 15, "invention an include" should read --invention can include--.
Line 61, "IN a rapid test" should read --In a rapid test--.

Signed and Sealed this

Twenty-eighth Day of November, 2006

JON W. DUDAS
Director of the United States Patent and Trademark Office

US006825040B2

(12) **United States Patent**
Scaringe et al.

(10) **Patent No.: US 6,825,040 B2**
(45) **Date of Patent: Nov. 30, 2004**

(54) **METHOD OF USING A VAPOR ACID TEST KIT**

(75) Inventors: **Robert P. Scaringe**, Rockledge, FL (US); **Nidal A. Samad**, Palm Bay, FL (US)

(73) Assignee: **Mainstream Engineering Corporation**, Rockledge, FL (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 7 days.

(21) Appl. No.: **10/282,725**

(22) Filed: **Oct. 30, 2002**

(65) **Prior Publication Data**

US 2003/0087451 A1 May 8, 2003

Related U.S. Application Data

(62) Division of application No. 08/423,211, filed on Apr. 17, 1995, now Pat. No. 6,514,765.

(51) **Int. Cl.**[7] **G01N 33/18**

(52) **U.S. Cl.** **436/39**; 436/163; 436/169; 422/58; 422/61; 422/86; 422/104

(58) **Field of Search** 422/58, 61, 86, 422/104; 436/39, 163, 164, 169

(56) **References Cited**

U.S. PATENT DOCUMENTS

3,084,658	A		4/1963	Schell
3,837,809	A	*	9/1974	Chapman 422/57
4,789,638	A	*	12/1988	Kramer et al. 436/111
4,923,806	A	*	5/1990	Klodowski 422/86
5,071,768	A		12/1991	Klodowski
5,127,433	A		7/1992	Argyle et al.
5,171,536	A	*	12/1992	Evers 422/88
5,363,661	A	*	11/1994	Condit et al. 62/77
5,377,496	A		1/1995	Otto et al.
6,514,765	B1	*	2/2003	Scaringe et al. 436/39

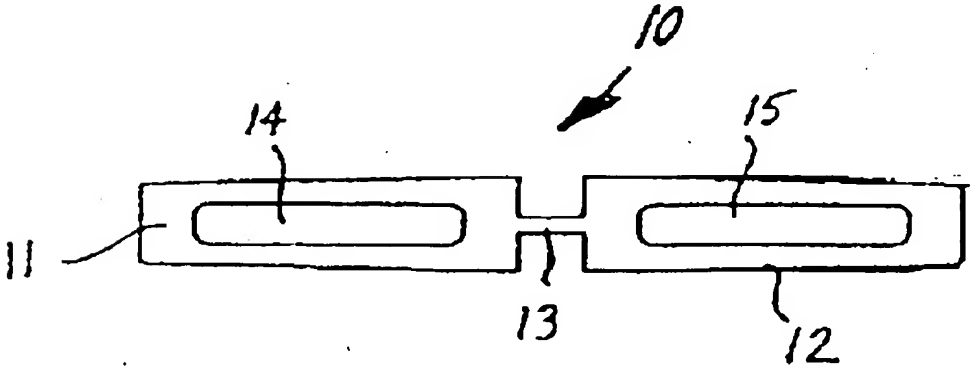
* cited by examiner

Primary Examiner—Lyle A. Alexander
(74) *Attorney, Agent, or Firm*—Crowell & Moring LLP

(57) **ABSTRACT**

An acid test kit uses an indicator paper in a transparent tube which is easily and temporarily inserted in a Schrader-valve at a compressor suction inlet of a refrigeration system. The simplicity, yet sufficient accuracy, of the kit permits several different ways of holding the paper in the tube through a friction fit. One way is to sandwich the paper between a tapered holder which is folded to provide a nose for actuating the Schrader valve. Another way is to provide the paper on or over a cylindrical member. A method for using the test kit including allowing vapor refrigerant from the system to pass through the tube to the atmosphere.

1 Claim, 6 Drawing Sheets



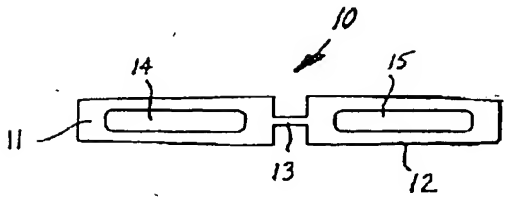


Fig. 1

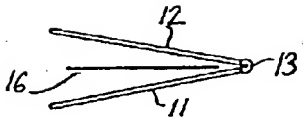


Fig. 2

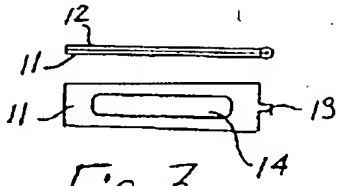


Fig. 3

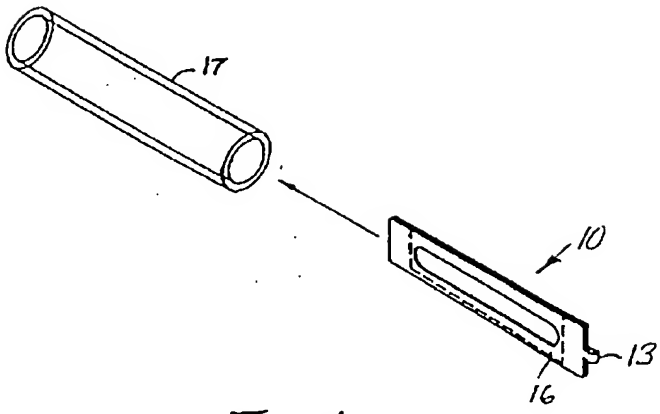


Fig. 4

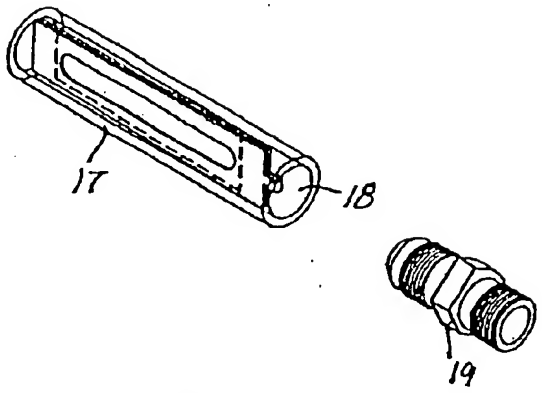


FIG. 5

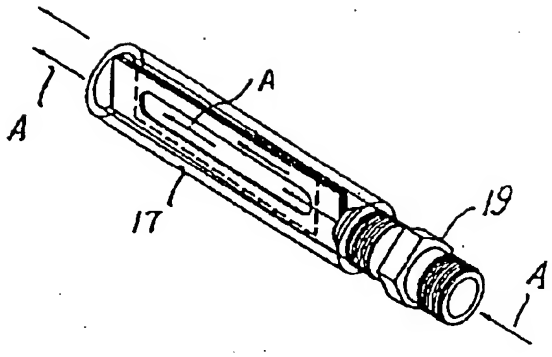


FIG. 6

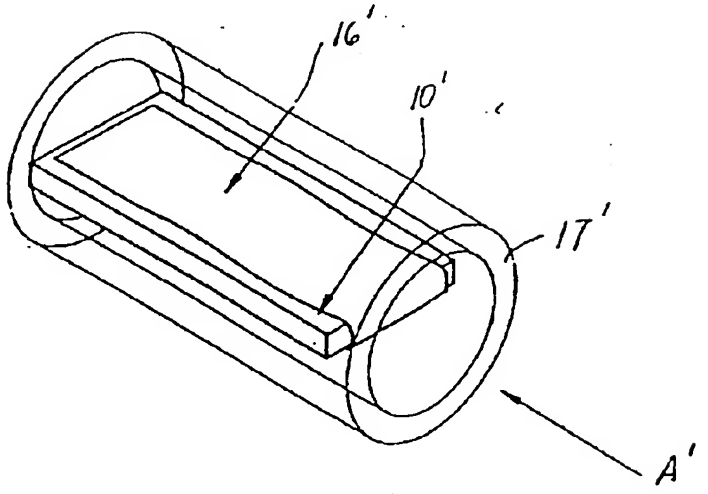


FIG. 7

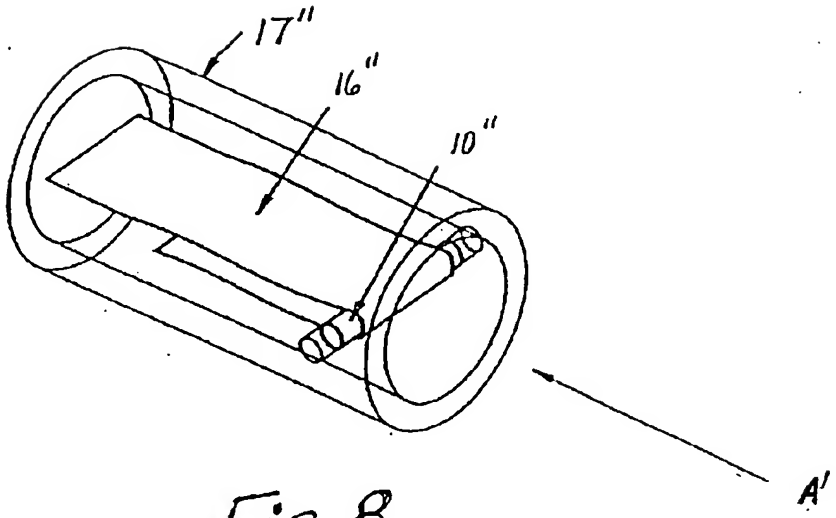


FIG 8

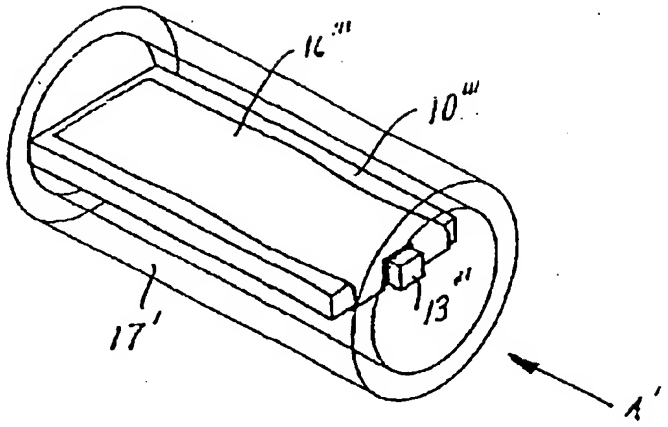


FIG. 9

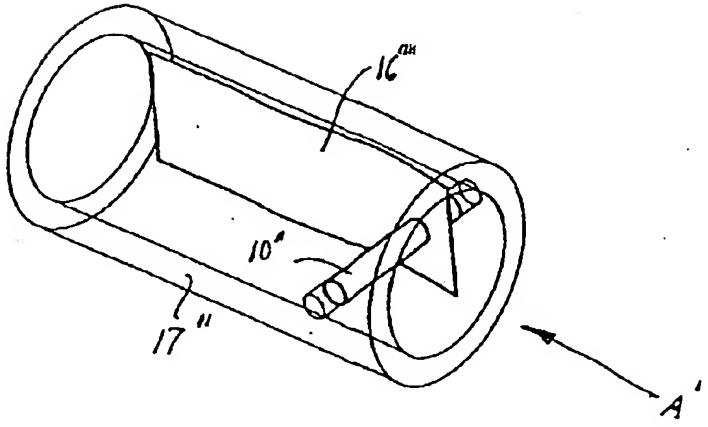


FIG. 10

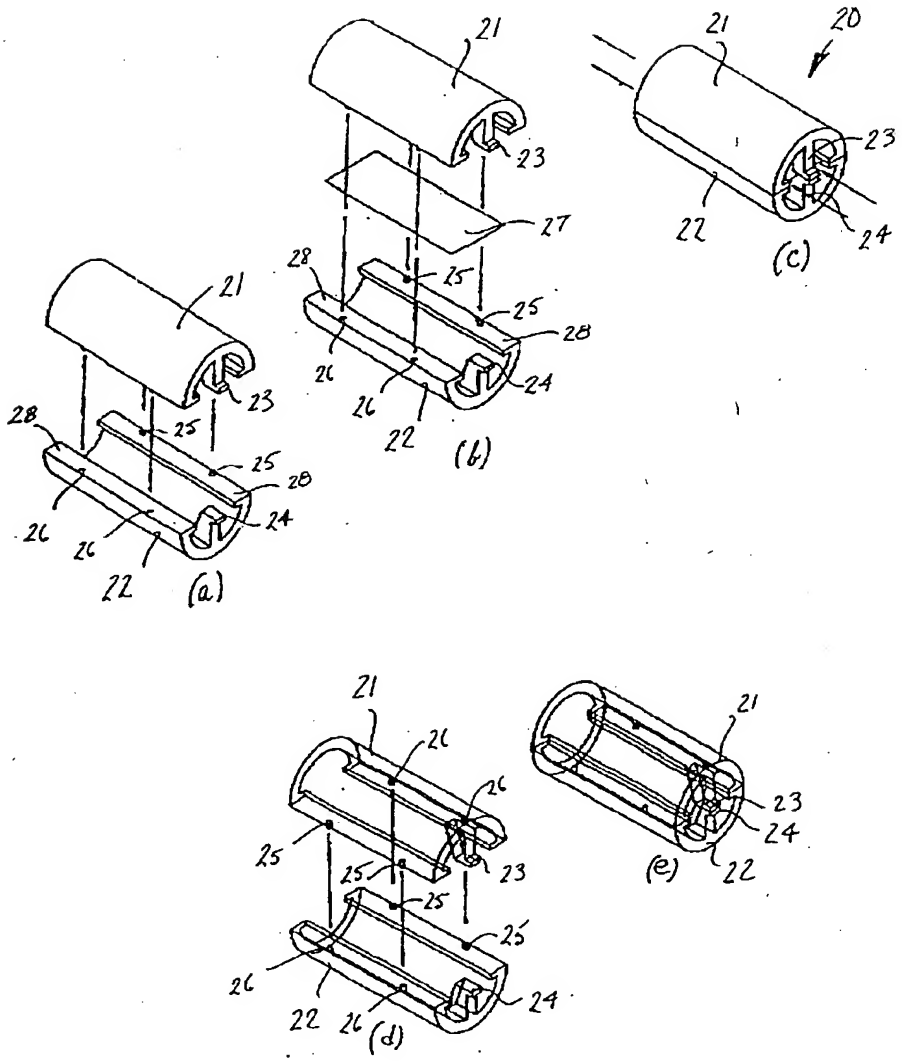

21
23
27
28
25
26
24
22
(b)
21
20
23
22
24
(c)
21
28
25
26
24
22
(a)
21
26
25
23
24
22
26
(d)
21
23
24
22
(e)

FIG. 11

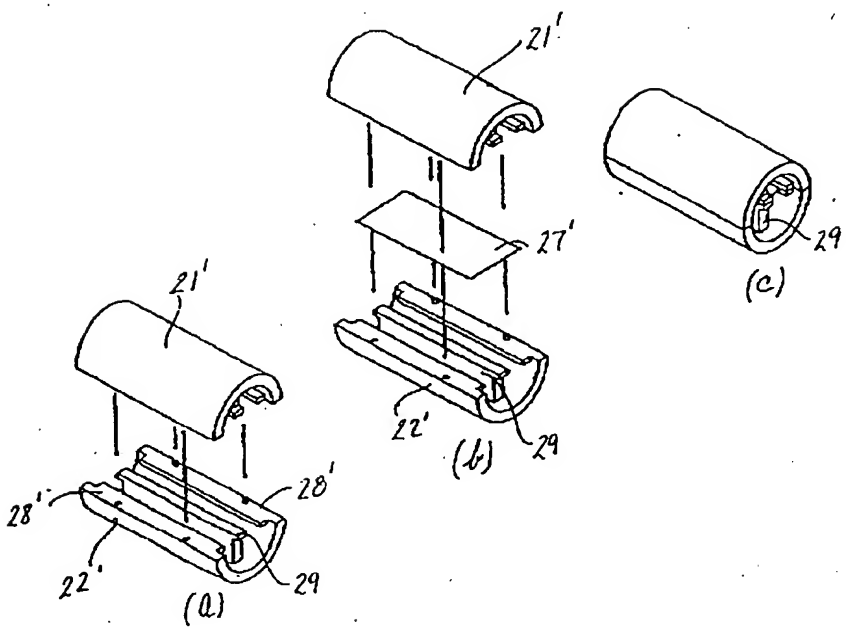


FIG. 12

METHOD OF USING A VAPOR ACID TEST KIT

This application is a divisional of application Ser. No. 08/423,211, filed Apr. 17, 1995 now U.S. Pat. No. 6,514,765.

BACKGROUND AND SUMMARY OF THE INVENTION

The present invention relates to an acid test kit and, more particularly, to an acid test kit used in vapor compression refrigerators and the like in which an indicator paper is held in a transparent tube-like fixture to monitor the acid level in the system simply, quickly and inexpensively.

Vapor compression refrigerators, heat pumps, and air conditioners must always be concerned with the presence of acids in the refrigerant which can severely shorten the life of both the compressor and the refrigerant. These acids can be formed by chemical reactions with components and/or materials of construction, lubricating oils, and/or impurities. The instability of the refrigeration, and thus the formation of acids, is accelerated by elevated temperatures which result from improper operation, such as a failed condenser fan, or clogged air flow path.

Checking the refrigerant and/or oil acid is a common maintenance procedure because acidic refrigerant can be cleaned up before permanent damage to the hardware and refrigerant occur. Acidic refrigerant will also result in hermetic compressor motor burn-out because the acid will degrade the motor winding's electrical insulation. Moreover, the presence of acid indicates the existence of other decomposition products, such as non-condensable gases, which result in elevated pressures and increased compressor pressure ratios leading to reduced efficiency and overloaded compressor operation.

To avoid the above-mentioned problems, refrigeration systems are tested for acid content. Typically, the oil would be tested for acid, because the highest concentration of acid is found in the oil of a non-operating system (shut down). It is, however, much easier to test the refrigerant for acid instead of testing the oil for acid, since the refrigerant is pressurized and existing service valves provide an easy way of sampling the refrigerant. Testing of the refrigerant vapor, rather than the refrigerant liquid, of the system is much more convenient because testing the liquid refrigerant results in a much greater refrigerant loss and the exiting liquid will cause frostbite if not properly handled.

Visual sensors or indicators for use in detecting the corrosive state of a fluid in a heat exchanger system are known as seen, for example, in U.S. Pat. No. 5,127,433. A permanently installed sensor has a sight glass or window through which corrosiveness is determined by viewing a flap or ball displaying a color indicating either the need to change the fluid or to add corrosion inhibitors. Alternatively, corrosiveness can be indicated by a ruptured or broken diaphragm located between the sight glass and the fluid. This form of sensor is limited to applications such as automobile cooling systems where the sensor is provided in the overflow conduit or in the hot fluid conduit upstream of the radiator.

Humidity and corrosion indicators for packaged goods in which thin cobaltous chloride film is used as the sensing element are described in U.S. Pat. No. 3,084,658. An elastomeric grommet sealed by a transparent disk is inserted into an opening in a package wall. A disk impregnated with the cobaltous chloride is secured beneath a window and can be replaced.

With respect to closed refrigeration systems, other types of indicator systems are known for testing the presence and concentration of contaminants in a refrigerant. For example, U.S. Pat. Nos. 4,923,806 and 5,071,768 show apparatuses for testing liquid or vapor contaminants in a closed system regardless of whether the apparatus is operating or not. A disposable testing tube made of transparent material is used at the end of a compressor discharge line or elsewhere in the system. One section of the tube is provided with water removal and moisture indicating chemicals, such as cobaltous chloride and another section is provided with acid indicating chemicals such as a solution of bromophenol blue, ethanol and glycerol. This construction is relatively complicated and requires a separate, specially configured flow restrictor in addition to a tube holder, and an expensive testing tube in which the multiple contaminant testing chemicals and filter screens are permanently located.

Likewise, U.S. Pat. No. 5,377,496 shows an acid contamination indicator for closed loop vapor compression refrigeration systems in which the indicator is permanently or removably installed in the bypass line around the system compressor where the refrigerant is always in the gaseous phase. A casing has a visual indicator bed of bromophenol blue as the acid indicating medium which is contacted by the refrigerant after flowing through a filter and a flow restrictor orifice. Porous retainer disks are held against the bed by springs. Moreover, the indicator, which changes color when exposed to acids or bases, are solid, and thus they must be exposed to the test stream in some fashion. Accordingly, this solid indicator must be mixed with an inert substance to provide some porosity, contact surface area and increased volume and then packaged in a clear tube. The vapor refrigerant is then passed through the porous mixture arranged in a bypass loop between the suction and discharge ends of a compressor or in the main refrigerant flow path between the compressor discharge and a heat exchanger to observe a color change. Again, we have recognized that this is an unduly complicated construction which requires a substantial outlay for installation.

Another type of contaminant detector is marketed by Refrigeration Technologies of Fullerton, Calif. under the trademark "CHECKMATE". A specific volume of gas passes through a detection tube at a predetermined termination pressure. However, an expensive sealed Pyrex detection tube containing a color-changing chemical and whose ends are pierced when fully assembled can only be used once even when the test is negative, and thus this approach entails considerable expense regardless of its technical merits.

In a vapor-compression system, refrigerant flows from the condenser to the expansion valve, where it flashes into a two-phase mixture and then enters the evaporator. Superheated refrigerant vapor, with some entrained oil, leaves the evaporator and is compressed in the compressor, before being condensed in the condenser to complete the cycle. When in chemical equilibrium, the majority of the acid in

the system is contained in the oil, but acid is also present in the liquid and vapor refrigerant. The presence of water in the system, which is a very real possibility, causes an even greater concentration of the acid in the liquid rather than in the vapor. To further complicate the problem, the relative liquid and vapor acid concentrations are a function of the system's liquid and vapor volume and therefore are system dependent. These factors all render the measurement of acid level in the refrigerant's equilibrium vapor phase an uncertain indication of acid level in the compressor oil.

Although the acid content in the refrigerant vapor can not be exactly correlated to the compressor oil acid content, it is, however, accurate enough to indicate the relative status of the oil in the system. It is clear that when acid is detected in the vapor, the acid level in the oil is significantly higher.

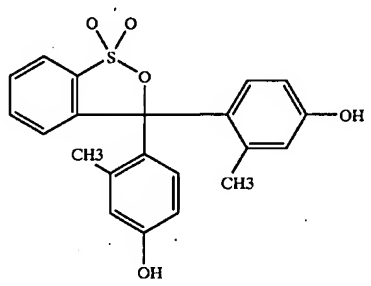
We have discovered that a pH paper can provide a simple fast and extremely inexpensive way to test for acidity in a refrigeration system. It allows for testing with the system on or off, and in other applications as well. In addition, it does not have to be installed in a line but can be temporarily connected with, for example, a Schrader-valve to permit venting of a small amount of gaseous refrigerant to the atmosphere. This is a surprising discovery because a pH paper is typically used to measure the concentration of hydronium ions in an aqueous solution. However, an aqueous solution is not present in a refrigerant system. Table 5–30 of *Lange's Handbook of Chemistry* (13th Edition) lists several chemical compounds for colorimetric pH indicators as follows:

Indicator	Chemical Name	pH Range	pK_a	λ_{max} nm	Color Change
Cresol red (acid range)	o-Cresolsulfonephthalein	0.2 to 1.8			R-Y
Cresol purple (acid range)	m-Cresolsulfonephthalein	1.2 to 2.8	1.51	533, . . .	R-Y
Thymol blue (acid range)	Thymolsulfonephthalein	1.2 to 2.8	1.65	544, 430	R-Y
Tropeolin 00	Diphenylamino-p-benzene sodium sulfonate	1.3 to 3.2	2.0	627, . . .	R-Y
2,5-Dinitrophenol	2,8-Dinitrophenol	2.4 to 4.0	3.69		C-Y
2,4-Dinitrophenol	2,4-Dinitrophenol	2.5 to 4.3	3.90		C-Y
Methyl yellow	Dimethylaminoazobenzene	2.9 to 4.0	3.3	508, . . .	R-Y
Methyl orange	Dimethylaminoazobenzene sodium sulfonate	3.1 to 4.4	3.40	622, 464	R-O
Bromophenol blue	Tetrabromophenolsulfonephthalein	3.0 to 4.8	3.85	436, 592	Y-BV
Bromocresol green	Tetrabromo-m-cresolsulfonephthalein	4.0 to 6.8	4.58	444, 617	Y-B
Methyl red	o-Carboxybenzeneazodimethylaniline	4.4 to 6.2	4.95	530, 427	R-Y
Chlorophenol red	Dichlorophenolsulfonephthalein	5.4 to 6.8	6.0	. . ., 573	Y-R
Bromocresol purple	Dibromo-o-cresolsulfonephthalein	5.2 to 6.8	6.3	433, 591	Y-P
Bromophenol red	Dibromophenolsulfonephthalein	5.2 to 6.8		. . ., 574	Y-R
p-Nitrophenol	p-Nitrophenol	5.3 to 7.6	7.15	320, 405	C-Y
Bromothymol blue	Dibromothymolsulfonephthalein	6.2 to 7.6	7.1	433, 517	Y-B
Neutral red	Aminodimethylaminotoluphenazonium chloride	6.8 to 8.0	7.4		R-Y
Phenol red	Phenolsulfonephthalein	6.4 to 8.0	7.9	433, 558	Y-M
m-Nitrophenol	m-Nitrophenol	6.4 to 8.8	8.3	. . ., 570	C-Y
Cresol red	o-Cresolsulfonephthalein	7.2 to 8.8	8.2	434, 572	Y-R
m-Cresol purple	m-Cresolsulfonephthalein	7.6 to 9.2	8.32	. . ., 580	Y-P
Thymol blue	Thymolesulfonephthalein	8.0 to 9.6	8.9	430, 596	Y-B
Phenolphthalein	Phenolphthalein	8.0 to 10.0	9.4	. . ., 553	C-R
α-Naphtholbenzein	α-Naphtholbenzein	9.0 to 11.0			Y-B
Thymolphthalein	Thymolphthalein	9.4 to 10.6	10.0	. . ., 598	C-B
Altzarin Yellow R	5-(p-Nitrophenylazo)-salicylic acid. Na salt	10.0 to 12.0	11.16		Y-V
Tropeolin 0	p-Sulfobenzeneazo-resorcinol	11.0 to 13.0			Y-O Br
Nitramine	2,4,6-Trinitrophenylmethylnitroamine	10.8 to 13.0			C-O Br

Independent of the system, it can be generally stated that a refrigerant vapor acid level about 1–2 parts per million (ppm) in the refrigerant vapor clearly means the oil acid content is high, and the system should be cleaned up to reduce the acid level. Furthermore, a refrigerant vapor acid of 10 ppm, clearly indicates the compressor's oil acid level is well beyond safe operating levels and the system will fail shortly if the refrigerant and acid is not changed or cleaned.

These compounds change color depending on the form they take (for example, yellow when acidic and blue when basic). A pH paper is a filter paper totally impregnated with one or more of these indicator compounds, generally an organic compound, that is a weak acid with a certain pKa (pKa is defined as the negative log of the dissociation equilibrium constant). The pKa value determines the range of the indicator.

Specifically for our invention, we currently contemplate use of a pH paper manufactured by Micro Essential Laboratory, Brooklyn, N.Y. with a pH range of 1–6. This pH paper is impregnated with meta-cresol purple (trade name) also known as meta-cresolsulfonephthalein (chemical name). The structure of this chemical is given below:



This indicator, meta-cresol purple, is red in acidic form and yellow in the basic form.

Our invention does not measure pH because the systems and the like with which the acid test kit is intended to be used do not contain an aqueous solution. We utilize pH paper because of the surprising discovery that it has an indicator which will react with the inorganic acid vapor present in the refrigeration system. Furthermore, filter paper impregnated with indicator solution is a commercially available product, namely pH paper, thus lowering the cost even more.

Thus, the present invention takes advantage of the low cost and ready availability of pH paper. If the indicator is in an acidic environment, the indicator will react with the acid and produce a red color. If the acid concentration is not enough to turn the indicator completely red, however, an intermediate color, between red and yellow (that is some shade of orange) will be observed. Therefore, the intensity of the color is concentration dependent. On the pH paper, a certain amount of the indicator is impregnated, and as the acid reacts with the indicator, the indicator's color changes. When most of the indicator has reacted, a red color will be observed. Therefore, the intensity of the color change depends on what percentage of the indicator has been transformed (reacted) to the acidic form.

We have also found that in a refrigeration system the refrigerant vapor acid test should be performed from the suction (vapor) service port. If both a compressor suction-side and compressor discharge-side vapor connection is available, we have further recognized that the lower-pressure suction side should be used and the system should be operating in order to minimize the amount of oil discharged with the refrigerant vapor and will also serve to provide a more acidic sample. When the vapor-compression system is operating, the liquid refrigerant with some dissolved oil is vaporized in the evaporator, resulting in an acid vapor refrigerant flow with entrained liquid oil droplets.

The acid level of the vapor stream during operation is not at equilibrium but instead is essentially the same as the acid level of the liquid that the refrigerant was flashed or evaporated from. Because the vapor is safer and much easier to sample, the sampling of the vapor phase during system operation is a much easier, safer, faster and better approach, as long as the system is operating, and will provide essentially the same accuracy as sampling of the liquid. In other words, although the equilibrium concentration of the acid in the vapor is lower than the acid concentration in the liquid refrigerant when the system is off (and would, therefore, provide lower and inaccurate pH readings), when the system is running (i.e., compressor operating, refrigerant flowing), the liquid refrigerant with acid and dissolved oil is flashed (evaporated) into superheated refrigerant vapor and entrained oil, and the acid is carried along with this vapor so that the acid level of this vapor is essentially the same as the acid level in the liquid refrigerant. Nevertheless, the acid test kit can be used in a system which has been shut off or in relation to a tank of recovered refrigerant. For those non-operational systems which need to be repaired, the oil can be tested directly (or simply changed) while the system is being repaired and thus does require an acid indicator which is safe and easy to use.

It is, therefore, an object of the present invention to provide an accurate, yet simple and inexpensive, acid test device which can sample the refrigerant's acid level, by sampling the refrigerant vapor, from existing system service valves, or from a refrigerant recovery tank, to provide an indication of the condition of the refrigerant and therefore the condition of the system.

In order to determine the pH of the vapor sample, it is necessary for a known amount of refrigerant be used in the test. We have further developed a simple method of determining this known amount of refrigerant. All systems typically have service valves with valve core depressors (often referred to as Schrader-valves). These valves, like automobile tire-valves, are opened when a valve core is depressed, usually by the device being attached to the valve. For refrigeration systems, these types of service valves with valve core depressors are used in several standard sizes, with ¼" being the most common and ⅜", ½", and ⅝" also used. For a given valve size, these service valves have a known flow cross-section. In order to determine the pH of the vapor sample, it is necessary to use a known amount of refrigerant. The present invention also uses these service valves as the flow metering device. In addition, for a given refrigerant, the system pressures are known, from the saturation pressure temperature correlation for the refrigerant. Therefore the combination of the known cross-section orifice area (service valve cross-sectional flow area) and known pressure can be used to calibrate refrigerant flow and therefore to calibrate acid level with the time necessary to react the indicator, that is to obtain a specific color change on the indicator.

The present invention advantageously uses a readily available, inexpensive indicator paper held in a transparent tube-like fixture to monitor the acid level. Indicator chemistry reaction is essentially a function of acid level and exposure time in an essentially linear fashion. That is, half the acid level exposed for twice the time will result in the same indicator reaction. Therefore, as discussed above, the effect of refrigerant flow must be considered in determining acid level. The present invention uses a standard refrigeration service valve with valve-core depressor (Schrader-valve), an industry-standard service valve, which is already present in essentially all refrigeration systems, as the natural throttling or metering device on the system. The system pressure depends only on the system refrigerant, thus advantageously allowing performance tables to be developed for each refrigerant.

BRIEF DESCRIPTION OF THE DRAWINGS

These and other objects, features and advantages of the present invention will become more readily apparent from the following detailed description thereof when taken in conjunction with the accompanying drawings wherein:

FIG. 1 is a side view of a slightly tapered indicator paper holder of the present invention in an unfolded position;

FIG. 2 is a plan view of the holder of FIG. 1 but in a partially folded condition with a piece of indicator paper in between legs of the holder;

FIG. 3 shows a side view of the holder of FIGS. 1 and 2 and a top view thereof in the fully folded position;

FIG. 4 is a perspective view showing insertion of the fully folded holder of FIG. 3 in an external tube;

FIG. 5 is a perspective view of the assembled holder and external transparent tube constituting a test kit with indicator paper showing attachment of a service valve;

FIG. 6 is a perspective view of the assembled external tube and service valve of FIG. 5;

FIG. 7 is a side view similar to FIG. 5 but showing a second embodiment of indicator holder;

FIG. 8 is a view similar to FIG. 5 but showing a third embodiment of indicator holder;

FIG. 9 is a view similar to FIG. 5 but showing a fourth embodiment of indicator holder;

FIG. 10 is a view similar to FIG. 5 but showing a fifth embodiment of indicator holder;

FIG. 11 consists of exploded views (*a*)–(*e*) showing another test kit embodiment in accordance with the present invention; and

FIG. 12 consists of exploded views (*a*)–(*e*) showing yet another test kit embodiment in accordance with the present invention.

DETAILED DESCRIPTION OF THE DRAWINGS

Referring now to FIG. 1, the acid indicator for a test kit is indicated generally by reference numeral **10** and consists of a holder having two slightly tapering panel portions **11, 12** joined by a connecting strip **13** whose additional function is described below. Each of the panel portions **11, 12** has an elongated aperture **14, 15**, respectively, to expose a test paper to refrigerant vapor. A standard pH-type acid test paper **16** is pinched or sandwiched between the panel portions **11, 12** which are folded together as seen in FIG. **2** into the final folded position of FIG. **3** with the connecting stop **13** forming a projecting nose or dimple.

The acid indicator insert **10** fitted with the pH test paper **16** is then inserted into a clear tube **17** as seen in FIG. **4**. The small raised dimple or nose formed by the folded connecting strip **13** is arranged at the tube inlet **18** as seen in FIG. **5** and is advantageously used to depress a valve core of the system's vapor-service-valve **19** which allows refrigerant vapor to flow through the test kit **10** in the manner shown by the arrows A in FIG. **6** to contact the paper **16** exposed through the apertures **14, 15**. The external clear tube **17** directs the refrigerant vapor past the indicator paper **16**, and the refrigerant is then exhausted into ambient air. The total time for the test is less than 15 seconds. Refrigerant vapor, not liquid, is tested by holding the test kit **10** against the system's vapor service valve **19**, typically the valve on compressor suction-side and only when the compressor (not shown) is operating.

In one embodiment of the present invention, the folded insert **10** is fabricated from a plastic material colored to the same color as the un-reacted indicator paper **16** held therewithin. Using the above-mentioned pH paper having a range of pH of 1.2 to 2.8, for example, the unreacted paper is yellow and after reaction the paper color is orange or red. The test kit operator holds the test kit against the refrigeration system's vapor service Schrader-valve for a five second count. If the indicator paper remains yellow by comparing it with the surrounding yellow plastic panel portions **11, 12**, then the acid level is deemed to be below 1–2 ppm, and the refrigerant and oil in the system are considered acceptably uncontaminated.

If the indicator paper turns orange within the five second count, however, acid is deemed present in the refrigerant vapor, and therefore also in the oil, at an unacceptable level above safe operating conditions. The refrigerant should then be cleaned or replaced. If recovered refrigerant is being tested, the refrigerant should be recycled to an acceptable acid level before using in a system. If the indicator paper **16** turns red, there is a very high acid level in the refrigerant vapor and the oil. Both the oil and refrigerant should be changed immediately, otherwise system failure is imminent, and this refrigerant should not be recycled. Finally, if the indicator paper **16** remains yellow, the acid test kit can be exposed to refrigerant for another five seconds while the system is running. If the indicator paper **16** still remains yellow, the acid content is very low, below 0.5–1.0 PPM. If, however, the indicator turns orange or red, clean up procedures should still be instituted.

A more accurate, but also somewhat more expensive, alternative approach is to color the plastic indicator holder **10**, for example, orange for the type of pH paper used in this embodiment. The test kit user then holds the indicator **10** in the clear tube **17** on the service valve **19** and counts the number of seconds it takes for the paper indicator to turn the orange color of the surrounding plastic panel portions **11, 12**. The time it takes to turn the indicator **10** to the specified color (that is the color of the plastic holder), is compared to a table for the particular refrigerant being tested to determine the acid content of the refrigerant. The test kit is configured so that if no color change occurs after fifteen seconds the test is stopped because essentially no acid is present.

Several other configurations of indicators can be used as shown in FIGS. **7** through **10**, where parts similar in function to the parts in FIGS. **1–6** are designated by the same numerals but primed. There are also several other standard indicator solutions, such as bromophenol blue and meta-cresol purple, which can be used for acid testing. Ordinary pH paper, which is typically used only for acid tests in aqueous solutions, has been found to work very well in the present invention because the results are repeatable, accurate, and fast. Alternatively, any standard indicator solution can also be used directly on the test kit's insert **10** by configuring the panel portions **11, 12** of paper which is directly treated with indicator chemistry. A currently contemplated embodiment is that shown in FIGS. **1–6** and contemplates using commercially available pH paper sandwiched into the color-coded bracket **10** which is slipped into the clear, slightly elastic tube **17** which is sized to be deformed into an oval cross-section upon insertion of the slightly tapered paper holder **10** into the tube **17** to insure a tight friction fit.

In the embodiment of FIG. **7**, the holder **10'** is a solid piece over which a piece of indicator paper **16'** is laid before insertion into the tube **17'**. In the embodiment of FIG. **8**, the holder **10"** is in the form of a cylindrical member installed in the clear tube **17"** such that a piece of indicator paper **16"** can be wrapped over the holder **10"**. The embodiment of FIG. **9** is similar to the embodiment of FIG. **7**, except that the holder **10'"** has a projecting nose **13'"** similar in function to the nose or dimple used in the embodiment in FIGS. **1–6**. The indicator paper **16'"** has an opening through which the nose **13'"** extends. The embodiment of FIG. **10** is similar to

the embodiment of FIG. **8** except that the indicator paper **16'''** has an aperture through which the cylindrical member **16'''** is inserted before insertion into the clear tube **17"**.

The indicator paper holder **10** can, for example, be fabricated from flat colored plastic or metal which is folded into the frame holding the indicator paper as shown in FIGS. **1–6**. This embodiment, which forms a rounded nose **13** has a wider surface for depressing the Schrader valve and allows use of a thinner material, such as 0.020". Because of the rounded nose **13**, however, the frontal area which depresses the Schrader-valve is much thicker, e.g. approximately 0.090" depending upon the material used and its ductility. This insert can also be slightly tapered along its long sides, as seen in FIG. **1**, to allow easier insertion into the clear somewhat elastic tube **17**, the width of the insert **10** being, for example, about 0.050" wider that the diameter of the tubing **17** to insure a tight friction fit.

A currently preferred embodiment of the present invention as shown in FIG. **11**, the test kit **20** is fabricated from two mating injection-molded and symmetrical parts **21, 22** molded from clear plastic material. Each half **21, 22** of the injected molded holder **20** has a raised dimple **23, 24** at the tube inlet which is used to depress the system's vapor-service-valve valve-core and allows refrigerant vapor to flow. On one side of both halves **21, 22** of the molded holder **20** are crush pins **25** which deform into mating holes **26** on the other half to lock the halves together as best seen in the views (*d*) and (*e*) in FIG. **11**. No glue or adhesive is used to avoid adversely affecting the pH paper **27**. A shelf or frame **28** is also molded into both halves **21, 22** to pinch and hold the indicator paper **27** in place.

To further simply the fabrication of the molded holder, a third embodiment of the present invention as seen in FIG. **12** is provided. To simplify fabrication, the raised dimple sections **23, 24** of FIG. **11** has been replaced by a complete center shelf **29** which also serves as an additional pinch point for the indicator paper. All other features of this embodiment remain the same as in FIG. **11** and therefore are designated by the same numerals but primed.

After fabrication, the acid test kit in each of the above-described embodiments is packaged in an airtight plastic bag to avoid contamination prior to use.

Although the invention has been described and illustrated in detail, it is to be clearly understood that the same is by way of illustration and example, and is not to be taken by way of limitation. The spirit and scope of the present invention are to be limited only by the terms of the appended claims.

We claim:

1. Method of using a sheet like pH-type indicator paper substrate in a test kit to react with inorganic acids in vapor compressor systems, comprising impregnating the paper substrate with a pH indicator selected from the group consisting of

σ-Cresolsultonephthalein
m-Cresolsultonephthalein
Thymolsulfonephthalein
Diphenylamino-p-benzene sodium sulfonate
2,6-Dinitrophenol
2,4,-Dinitrophenol
Dimethylamlnoazoben-zene
Dimethylamlnoazoben-zene sodium sulfonate
Tetrabromophenolsulfone-phthalein
Tetrabromo-m-cresol-sulfonephthalein
σCarboxybenzenoazo-dimethylanillne
Dichlorophenolsulfone-phthalein
Dibromo-o-cresolsulfone-phthalein
Dibromophernolsulfone-phthalein
p-Nitrophenol
Olbromothymolsulfone-phthalein
Aminodimathylaminotolu-phenazonium chloride
Phenolsulfonephthalein
m-Nitrophenoi
σ-Cresolsulfonephthalein
m-Cresolsulfonephthalein
Thymolsulfonephthalein
Phenolphthalein
σNaphtholbenzein
Thymolphthalein
δ-(p-Nitrophenylazo)-salicylic acid Na salt
p-Sulfobenzeneazo-resorcinol
2,4,5-Trinitrophanyl-methylnitroamine

and removably holding the sheet-like paper substrate in a hollow transparent member such that the held substrate is viewable therethrough, and open at upstream and downstream ends thereof so as to define a free cross-section disposed along a flow of vapor in the vapor compressor system through the member when the upstream end thereof is temporarily held against a service valve of a system for flowing the vapor over an outer surface of the sheet-like paper substrate into the atmosphere.

* * * * *

United States Patent [19]

Bäther

[11] **Patent Number: 4,844,867**

[45] **Date of Patent: Jul. 4, 1989**

[54] **COLORIMETRIC DETECTOR**

[75] Inventor: **Wolfgang Bäther, Lübeck, Fed. Rep. of Germany**

[73] Assignee: **Drägerwerk Aktiengesellschaft, Lübeck, Fed. Rep. of Germany**

[21] Appl. No.: **52,787**

[22] Filed: **May 20, 1987**

[30] **Foreign Application Priority Data**

May 21, 1986 [DE] Fed. Rep. of Germany 3617023

[51] **Int. Cl.**[4] **G01N 1/48; G01N 21/06**

[52] **U.S. Cl.** **422/60; 422/56; 422/57; 436/902**

[58] **Field of Search** 422/55, 56, 57, 58, 422/59, 60, 86, 87; 436/902, 50, 128, 130; 252/408.1; 568/488

[56] **References Cited**

U.S. PATENT DOCUMENTS

4,308,028	12/1981	Elkins	422/58
4,348,358	9/1982	McKee et al.	422/58
4,521,376	6/1985	Witonsky et al.	252/408.1
4,666,859	5/1987	Attar	436/902
4,687,529	8/1987	Wang	422/58

FOREIGN PATENT DOCUMENTS

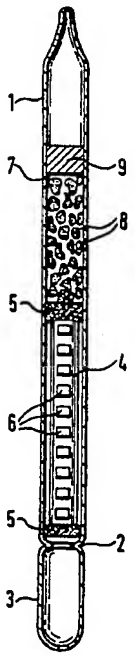
2084725	4/1982	United Kingdom .	

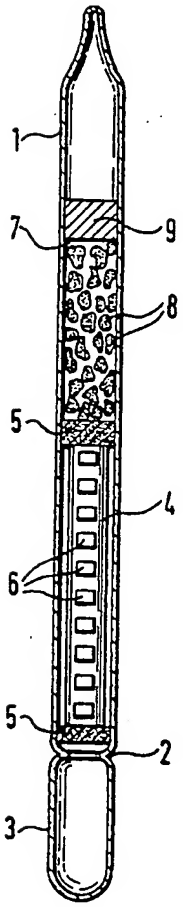
Primary Examiner—Barry S. Richman
Assistant Examiner—T. J. Wallen
Attorney, Agent, or Firm—Walter Ottesen

[57] **ABSTRACT**

A colorimetric detector such as a gas dosimeter, contains a strip-like carrier with a detection reagent in a transparent housing that can be opened at least at one end thereof. The detector is expanded in terms of its use to such reagent systems in which the pigment formed diffuses beyond the reaction zone. For this purpose, the detection reagent is provided such that it is contained in impregnated tabs arranged in a row on the carrier.

12 Claims, 1 Drawing Sheet




1
7
9
8
5
4
6
5
2
3

COLORIMETRIC DETECTOR

FIELD OF THE INVENTION

The invention relates to a colorimetric detector such as a gas dosimeter which contains a strip-like carrier with a detection reagent in a transparent housing. The housing can be opened on at least one end thereof.

BACKGROUND OF THE INVENTION

A gas dosimeter of this kind is disclosed in United Kingdom Pat. No. 2 084 725 B.

A strip impregnated with a detection reagent, when exposed to the gas that is to be detected, develops a color change which with sustained exposure time progresses along the strip. The colored length then represents the measure for the gas concentration that is to be detected.

Especially when measuring low concentrations of a toxic substance over a relatively long measuring period, such reagents, which provide a sensitive and specific color reaction with the gas to be detected but in which the pigment formed is soluble within the impregnation at a high diffusion rate, cannot be used as a length indicator, because instead of remaining as an indicator agent in the reaction zone, the pigment distributes out of the reaction zone into the zones where a reaction has not yet occurred, making a length reading impossible. If required, an evaluation would be possible with respect to the color saturation, but without auxiliary aids this is even more difficult and less accurate than a length reading.

SUMMARY OF THE INVENTION

It is an object of the invention to extend the use of the known gas dosimeter to such reagent systems in which the pigment formed diffuses beyond the reaction zone.

The object is attained by providing that the detection reagent is contained in impregnated tabs disposed in a row on the carrier.

The advantages attained with the invention are substantially that the tabs can be simply attached to the carrier, for example, by applying the suitably dissolved detection reagent with a pipette on fields that are equipped so as to be hydrophilic. Since the individual tabs are separated from one another by non-hydrophilic intervening spaces, a change in color of the detection reagent is restricted to one tab, and the change in color cannot spread to the next tab by mere diffusion of the reaction products within the detection reagent. Instead, it is assured that the change in color of the individual tabs can be essentially ascribed only to the action of the toxic substance.

The dimensioning and shape of the individual tabs can easily be adapted to the accuracy of the reading required. For insensitive dosimeters, for example, relatively large square tabs may be provided, whereas in contrast thereto and to increase the sensitivity, a closely spaced succession of minimum-sized tabs disposed transversely to the direction of diffusion and spaced apart by the least possible distance from one another can be provided.

By means of a succession of tabs having a different surface area, that is, a different length and/or width, instruments having inherently different sensitivity and thus a very wide measurement range can be provided; in the evaluation, tabs of different size are weighted differently.

Since the number of tabs that have changed color is now the measure of the toxic substance concentration, it is not necessary to provide a measuring scale on the housing. As a result, the costly alignment of the measuring scale with the position of the reagent layer can be dispensed with.

With the detector according to the invention, reagents which in the known equipment can be used only for a comparative measurement of depth of color can thus be used for a length measurement of the color change zone.

Advantageously, such reagents, which are present in a dissolved form, can be contained in the tabs. The tabs are easily applied onto the hydrophilic fields; because they are distributed among tabs they are retained over the entire area of the carrier in terms of their surface distribution, and in terms of a color reaction they prove to be particularly reactive.

For the case where the individual tabs have a very small amount of detection reagent applied, it is advantageous to provide a buffer chamber in the dosimeter housing, which emits volatile ingredients that could diffuse out of the tabs into the reagent space. In this way, a constant composition of the individual reagent ingredients in the tabs is assured and the detection reagents will not dry out during relatively long periods of storage.

During the use of the gas dosimeter as well, that is when the housing is opened, the volatile components of the reagent system are replenished, so that the state of equilibrium required for the detection reaction remains as at the outset.

For reagent systems that cause undesirable reactions under the influence of air during storage, it is advantageous to fill the dosimeter housing with an inert gas. This may for example be nitrogen or an oxygen-free gas mixture.

The carrier is advantageously a polyester foil which is easily impregnated with individual tabs.

Cellulose has proved to be a suitable base for the tabs. Other tab materials can, for example, be silica gel or aluminum oxide. The gas dosimeter is highly suitable for detecting formaldehyde, with the tabs being impregnated with a pararosaniline-hydrogen chloride-sulfite reagent. The course of the detection reaction in the tabs is that pararosaniline acid, catalyzed with formaldehyde, reacts to form an imine, to which sulfite ions become added, which form a sulfonic acid derivative and finally form a blue pigment.

To prevent drying out of the detection reagent, it is suitable to admix a hydrocolloid as a moisture stabilizer. This may for example comprise gelatine or pectin.

Since the sulfite formed during the pararosaniline reaction is converted by air oxidation into sulfuric acid, and sulfuric acid causes decomposition of the pigment, ascorbic acid is suitably added to the detection reagent as a sulfite oxidation inhibitor.

To prevent an air filling of the dosimeter housing causing chemical reactions in the detection reagent during the storage period, which would thwart a visible color reaction when the dosimeter is used, the dosimeter housing is advantageously filled with an oxygen-free inert gas. This is indicated particularly in the detection of formaldehyde, because during this long period, the sulfur dioxide in the reagent could oxidize, and the

resultant sulfuric acid could destroy the detection reagent.

In order to assure a constant consistency of the detection reagent during the storage period as well as during the period of use of the gas dosimeter, the buffer chamber is provided with a granulated filling of silicon dioxide, which is impregnated with a solution of hydrogen chloride, sodium sulfite and water. A buffer chamber of this kind should be provided particularly if the indication sensitivity is brought about by reduction of the reagent volume in the tabs. In such a case, even slight quantities of toxic substance can be enough for a complete color reaction. The small volume of reagent is then opposed by the largest possible diffusing gas volume. In that case, the small reagent volume is no longer able to compensate for the losses of volatile components.

BRIEF DESCRIPTION OF THE DRAWING

The single figure of the drawing shows a schematic of the colorimetric gas dosimeter according to an embodiment of the invention.

DESCRIPTION OF THE PREFERRED EMBODIMENT OF THE INVENTION

The schematic shows the colorimetric gas dosimeter in a transparent tubular glass housing **1**. One of its ends can be opened with a cap **3** that can be broken off at the constriction **2**. A strip-like carrier **4** is received between two permeable holder elements **5**, facing the openable end, in the housing **1**. A plurality of square tabs **6** are mounted in succession on the carrier **4**. They contain the reagent necessary for the gas measurement.

A buffer chamber **7** is provided and contains a filling **8** that is fixed in its position by means of a plug **9**. The buffer chamber **7** is disposed behind the carrier **4**.

It is understood that the foregoing description is that of the preferred embodiments of the invention and that various changes and modifications may be made thereto without departing from the spirit and scope of the invention as defined in the appended claims.

What is claimed is:

1. A colorimetric gas dosimeter for measuring a quantity of a gaseous substance, the colorimetric gas dosimeter comprising:

a transparent elongated closed housing having a predetermined length;

said housing having an end portion and a housing wall;

means formed in said wall for facilitating a separation of said end portion from said housing so as to provide an opening in said housing for receiving a gaseous substance to be measured into said housing;

a strip-like carrier defining a longitudinal axis and mounted in said housing so as to extend along said length thereof;

a plurality of tabs arranged in a row on said carrier and each of said tabs containing a detecting reagent for reacting with said substance to yield reaction products which produce a change in color;

each of said tabs and the reagent contained therein corresponding to a specific level of concentration of said substance whereby the number of tabs that have changed color is a measure of the quantity of said substance which is present; and,

each two mutually adjacent ones of said tabs being separated from each other by a non-hydrophilic unobstructed space to prevent said reaction products from diffusing from one of said tabs to the next adjacent one of said tabs.

2. The colorimetric detecting arrangement of claim **1**, said detecting reagent being in a dissolved form.

3. The colorimetric detecting arrangement of claim **1**, said closed housing being filled with an inert gas.

4. The colorimetric detecting arrangement of claim **1**, said strip-like carrier being made of a polyester foil.

5. The colorimetric detecting arrangement of claim **1**, said strip-like carrier having a coating of cellulose material formed thereon in the region of said tabs.

6. The colorimetric detecting arrangement of claim **1**, said tabs being impregnated with said detecting reagent and the latter being a pararosaniline-hydrogen chloride-sulfite reagent for detecting formaldehyde.

7. The colorimetric detecting arrangement of claim **6**, wherein a hydrocolloid is admixed to said detecting reagent as a humidity stabilizer.

8. The colorimetric detecting arrangement of claim **6**, wherein ascorbic acid is added to said detecting reagent as a sulfite-oxidation inhibitor.

9. The colorimetric detecting arrangement of claim **6**, wherein the housing is filled with an inert gas in the form of an oxygen-free atmosphere.

10. A colorimetric gas dosimeter for measuring a quantity of a gaseous substance, the colorimetric gas dosimeter comprising:

a transparent elongated closed housing having a predetermined length;

said housing having an end portion and a housing wall;

means formed in said wall for facilitating the separation of said end portion so as to provide an opening in said housing for receiving a gaseous substance to be measured into said housing;

a strip-like carrier defining a longitudinal axis and mounted in said housing so as to extend along said length thereof;

a plurality of tabs arranged in a row on said carrier and each of said tabs containing a detecting reagent for reacting with said substance to produce a change in color and reaction products; and,

said detecting reagent includes volatile constituents and said arrangement further comprises a gas permeable partition interface for partitioning of said housing into first and second elongated chambers disposed one behind the other along the length of said housing; said strip-like carrier being mounted in said first chamber and said second chamber being a buffer chamber containing volatile substance means for releasing the same into said interior of said housing for assuring a constant composition of the reagent, said volatile substance means being in equilibrium with said volatile constituents of said reagent.

11. The colorimetric detecting arrangement of claim **10**, wherein said buffer chamber contains a granular charge of silicon dioxide impregnated with a hydrogen chloride-sodium sulfite-water solution.

12. The colorimetric detecting arrangement of claim **3**, wherein each two mutually adjacent ones of said tabs are spaced apart a predetermined distance to prevent said reaction products from diffusing from one of said tabs to the next adjacent one of said tabs.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 4,844,867

DATED : July 4, 1989

INVENTOR(S) : Wolfgang Bäther

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In column 4, line 64: delete "3, wherein" and substitute -- 10, wherein -- therefor.

Signed and Sealed this

Tenth Day of April, 1990

Attest:

Attesting Officer

HARRY F. MANBECK, JR.

Commissioner of Patents and Trademarks

DOCUMENT-IDENTIFIER: US 4844867 A
** See image for Certificate of Correction **
TITLE: Colorimetric detector

Brief Summary Text (20):
Cellulose has proved to be a suitable base for the tabs. Other tab materials can, for example, be silica gel or aluminum oxide. The gas dosimeter is highly suitable for detecting formaldehyde, with the tabs being impregnated with a pararosaniline-hydrogen chloride-sulfite reagent. The course of the detection reaction in the tabs is that pararosaniline acid, catalyzed with formaldehyde, reacts to form an imine, to which sulfite ions become added, which form a sulfonic acid derivative and finally form a blue pigment.

US 20050085739A1

(19) United States

(12) Patent Application Publication (10) Pub. No.: US 2005/0085739 A1

MacDonald et al. (43) Pub. Date: Apr. 21, 2005

(54) VISUAL INDICATING DEVICE FOR BAD BREATH

(75) Inventors: John Gavin MacDonald, Decatur, GA (US); Yanbin Huang, Roswell, GA (US); Kevin Peter McGrath, Alpharetta, GA (US); RameshBabu Boga, Roswell, GA (US)

Correspondence Address:
KIMBERLY-CLARK WORLDWIDE, INC.
401 NORTH LAKE STREET
NEENAH, WI 54956

(73) Assignee: Kimberly-Clark Worldwide, Inc.

(21) Appl. No.: 10/687,270

(22) Filed: Oct. 16, 2003

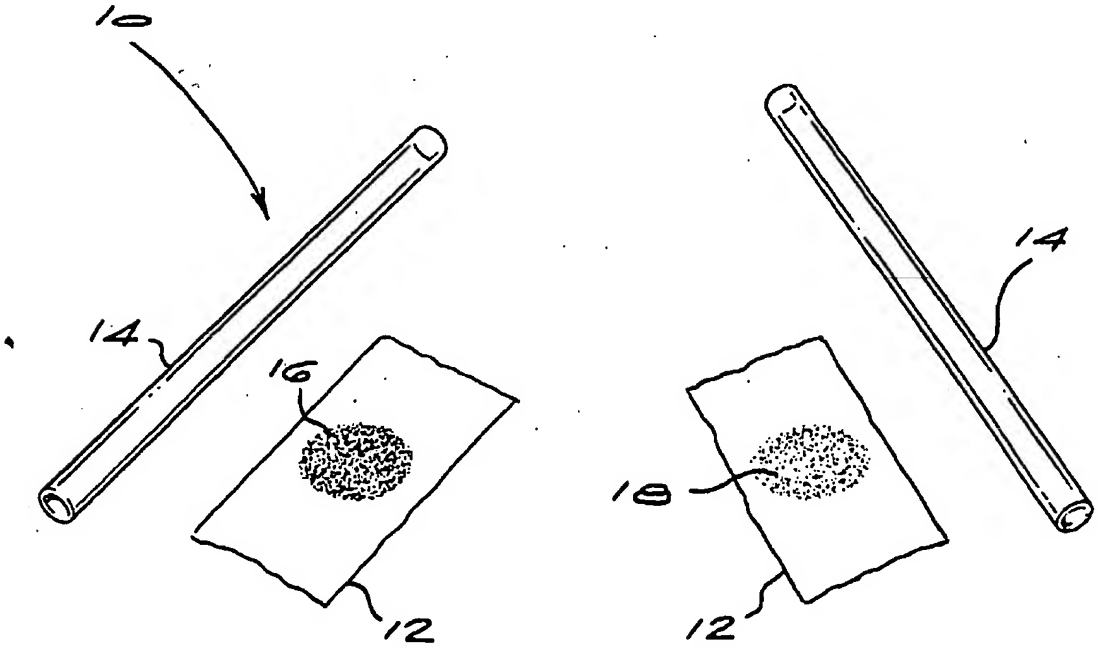
Publication Classification

(51) Int. Cl.[7] A61B 5/08

(52) U.S. Cl. 600/530

(57) ABSTRACT

The invention provides a breath testing device which includes a visual indicating agent which changes color in the presence of an odor associated with bad breath, such as sulfur and ammonia odors. An example of the visual indicating agent is 4,4'-bis(dimethylamino)-benzhydrol (Michler's hydrol or BDMB) and related dyes having a similar chemical structure. The indicating agent is applied to a substrate which is then inserted into a tube or straw, or which covers one end of a straw. When a user with bad breath blows into the tube or straw, the indicating agent will change color. The breath testing devices provide a quick and affordable means for a user to test their breath, and they may be packaged in discreet, pocket-sized dispensers which can be carried in a pocket or purse.



DOCUMENT-IDENTIFIER: US 20050085739 A1
TITLE: Visual indicating device for bad breath

Abstract Paragraph:
The invention provides a breath testing device which includes a visual indicating agent which changes color in the presence of an odor associated with bad breath, such as sulfur and ammonia odors. An example of the visual indicating agent is 4,4'-bis(dimethylamino)-benzhydrol (Michler's hydrol or BDMB) and related dyes having a similar chemical structure. The indicating agent is applied to a substrate which is then inserted into a tube or straw, or which covers one end of a straw. When a user with bad breath blows into the tube or straw, the indicating agent will change color. The breath testing devices provide a quick and affordable means for a user to test their breath, and they may be packaged in discreet, pocket-sized dispensers which can be carried in a pocket or purse.

CLAIMS:

28. The breath testing device of claim 23, wherein the visual indicating agent contains 4,4'-bis (dimethylamino)-benzhydrol.

US 20060003336A1

(19) United States

(12) Patent Application Publication (10) Pub. No.: US 2006/0003336 A1

Song et al. (43) Pub. Date: Jan. 5, 2006

(54) ONE-STEP ENZYMATIC AND AMINE DETECTION TECHNIQUE

(75) Inventors: Xuedong Song, Roswell, GA (US); RameshBabu Boga, Roswell, GA (US); Chibueze Obi Chidebelu-Eze, Atlanta, GA (US)

Correspondence Address:
Dority & Manning, P.A.
P.O. Box 1449
Greenville, SC 29601 (US)

(73) Assignee: Kimberly-Clark Worldwide, Inc.

(21) Appl. No.: 10/881,010

(22) Filed: Jun. 30, 2004

Publication Classification

(51) Int. Cl.

C12Q 1/68	(2006.01)
G01N 33/573	(2006.01)
G01N 33/53	(2006.01)

(52) U.S. Cl. 435/6; 435/7.92; 435/23

(57) ABSTRACT

A technique for detecting the presence or quantity of an enzyme (or enzyme inhibitor) and/or an amine within a test sample is provided. For example, in one embodiment, a diagnostic test kit is employed that utilizes reactive complexes that each includes a substrate joined (e.g., covalently bonded, physically adsorbed, etc.) to a reporter and a separation species. Upon contacting the reactive complexes, enzymes may cleave the substrate and release the reporter. Moreover, the test kit may also employ a chemichromic dye, i.e., a dye that exhibits a detectable color change upon chemical reaction with one or more functional groups, such as amino groups. The signal generated (directly or indirectly) by the reporter and chemichromic dye may then be used to indicate the presence or quantity of an enzyme (or enzyme inhibitor) and amine, respectively, within the test sample.

DOCUMENT-IDENTIFIER: US 20060003336 A1
TITLE: One-step enzymatic and amine detection technique

Description of Disclosure:
[0009] FIG. 1 is a perspective view of one embodiment of an assay device that may be used in the diagnostic test kit of the present invention;

Description of Disclosure:
[0040] As stated above, various separation techniques may be utilized in the present invention for separating any released reporters from unreacted complexes including, but not limited to, chemical separation techniques, magnetic separation techniques, etc. In one particular embodiment, for example, the diagnostic test kit contains an assay device that employs a chromatographic medium for separating unreacted complexes from released reporters. In contrast to other techniques, such as centrifugation, the use of a chromatographic medium may simplify and reduce the costs of the resulting diagnostic test kit for many consumer applications, including those in which a disposable kit is desired. Further, the use of a chromatographic medium also provides for a mechanism in which two different species, i.e., an enzyme (or inhibitor) and amine, may be simultaneously tested in a single step. That is, a user may use the kit to test a single sample for an enzyme (or inhibitor) and/or amine.

Description of Disclosure:
[0041] Referring to FIG. 1, for instance, one embodiment of an assay device 20 that may be used in the present invention will now be described in more detail. As shown, the assay device 20 contains a chromatographic medium 23 optionally carried by a support 21. The chromatographic medium 23 may be made from any of a variety of materials through which a fluid is capable of passing, such as a fluidic channel, porous membrane, etc. For example, the chromatographic medium 23 may be a porous membrane formed from materials such as, but not limited to, natural, synthetic, or naturally occurring materials that are synthetically modified, such as polysaccharides (e.g., cellulose materials such as paper and cellulose derivatives, such as cellulose acetate and nitrocellulose); polyether sulfone; polyethylene; nylon; polyvinylidene fluoride (PVDF); polyester; polypropylene; silica; inorganic materials, such as deactivated alumina, diatomaceous earth, MgSO.sub.4, or other inorganic finely divided material uniformly dispersed in a porous polymer matrix, with polymers such as vinyl chloride, vinyl chloride-propylene copolymer, and vinyl chloride-vinyl acetate copolymer; cloth, both naturally occurring (e.g., cotton) and synthetic (e.g., nylon or rayon); porous gels, such as silica gel, agarose, dextran, and gelatin; polymeric films, such as polyacrylamide; and so forth. In one particular embodiment, the chromatographic medium is formed from nitrocellulose and/or polyether sulfone materials. It should be understood that the term "nitrocellulose" refers to nitric acid esters of cellulose, which may be nitrocellulose alone, or a mixed ester of nitric acid and other acids, such as aliphatic carboxylic acids having from 1 to 7 carbon atoms.

Description of Disclosure:
[0043] The assay device 20 may also contain an absorbent material 28. The absorbent material 28 generally receives fluid that has migrated through the entire chromatographic medium 23. As is well known in the art, the absorbent material 28 may assist in promoting capillary action and fluid flow through the medium 23. The assay device 20 may also include a sample pad 22 or other material that is in fluid communication with the chromatographic medium 23. Some suitable materials that may be used to form the sample pad 22 include, but are not limited to, nitrocellulose, cellulose, porous polyethylene pads, and glass fiber filter paper. If desired, the sample pad 22 may contain one or more assay pretreatment reagents, either diffusively or non-diffusively attached thereto.

Description of Disclosure:

[0044] Generally speaking, the manner in which the assay device 20 functions may depend on the type of separation species selected for the reactive complexes. In this regard, various techniques for using the assay device 20 in embodiments in which the separation species is a specific binding member will now be described in more detail. For example, as stated above, the reactive complexes are generally allowed to incubate with the test sample for a certain period of time. This incubation process may be conducted before applying the test sample to the chromatographic medium 23, or it may be incorporated as part of the assaying procedure (i.e., incubation occurs after the test sample is applied, such as within an incubation well). For instance, the incubation mixture may be directly applied to a portion of the chromatographic medium 23 through which it may then travel in the direction illustrated by arrow "L" in FIG. 1. Alternatively, the mixture may first be applied to the sample pad 22.

Description of Disclosure:
[0052] Of course, any other suitable technique for capturing and detection the released reporters may also be used. For example, in some embodiments, non-biological receptive materials may be immobilized within the second enzyme detection zone 35 for capturing released reporters. Such non-biological receptive materials may be particularly useful in capturing, for example, released reporters that contain labeled particles. For instance, in one embodiment, the receptive material is a polyelectrolyte. Polyelectrolytes may have a net positive or negative charge, as well as a net charge that is generally neutral. Some suitable examples of polyelectrolytes having a net positive charge include, but are not limited to, polylysine (commercially available from Sigma-Aldrich Chemical Co., Inc. of St. Louis, Mo.), polyethylenimine; epichlorohydrin-functionalized polyamines and/or polyamidoamines, such as poly(dimethylamine-co-epichlorohydrin); polydiallyidimethyl-ammonium chloride; cationic cellulose derivatives, such as cellulose copolymers or cellulose derivatives grafted with a quaternary ammonium water-soluble monomer; and so forth. In one particular embodiment, CelQuat.RTM. SC-230M or H-100 (available from National Starch & Chemical, Inc.), which are cellulosic derivatives containing a quaternary ammonium water-soluble monomer, may be utilized. Moreover, some suitable examples of polyelectrolytes having a net negative charge include, but are not limited to, polyacrylic acids, such as poly(ethylene-co-methacrylic acid, sodium salt), and so forth. It should also be understood that other polyelectrolytes may also be utilized in the present invention, such as amphiphilic polyelectrolytes (i.e., having polar and non-polar portions). For instance, some examples of suitable amphiphilic polyelectrolytes include, but are not limited to, poly(styryl-b-N-methyl 2-vinyl pyridinium iodide) and poly(styryl-b-acrylic acid), both of which are available from Polymer Source, Inc. of Dorval, Canada. Further examples of polyelectrolytes are described in more detail in U.S. Patent App. Publication No. 2003/0124739 to Song, et al., which is incorporated herein in it entirety by reference thereto for all purposes.

Description of Disclosure:
[0054] Besides using receptive materials, other capturing techniques may also be utilized. For example, in one embodiment, the reporter may contain a magnetic substance that is capable of being captured by a magnetic device. In one embodiment, the magnetic device is positioned adjacent to (e.g., below) the second enzyme detection zone 35 defined by the chromatographic medium 23. In this manner, the magnetic device may immobilize the released reporters, as well as any partially cleaved or unreacted complexes, within the second enzyme detection zone 35. Any magnetic device may be used in the present invention. A magnetic field generator, for instance, may be used to generate a magnetic field that elicits a response from the magnetic substances. Suitable magnetic field generators include, but are not limited to, permanent magnets and electromagnets. Some commercially available examples of suitable magnetic separation devices include the Dynal MPC series of separators manufactured by Dynal, Inc. of Lake Success, N.Y., which employ a permanent magnet located externally to a container holding a test medium. Still other magnetic devices may be described in U.S. Pat. No. 5,200,084 to Liberti, et al.; U.S. Pat. No. 5,647,994 to Tuunanen, et al.; U.S. Pat. No. 5,795,470 to Wang, et al.; and U.S. Pat. No. 6,033,574 to Siddigi, which are incorporated herein in their entirety by reference thereto for all

purposes.

Description of Disclosure:
[0058] The probes may be contacted with the released reporters at any stage of the enzyme detection process. For example, in some embodiments, the probes may be applied to the assay device 20 at a location upstream from the region in which detection is desired. For example, in one embodiment, the probes may be applied to a conjugated pad (not shown) that is located upstream from the enzyme detection zones 31 and 35, but downstream from the sample pad 22.

Description of Disclosure:
[0064] In the embodiments described above, magnetic separation of the released magnetic substance, partially cleaved reactive complexes, and unreacted complexes, may occur prior to assaying the released reporters. In some embodiments, however, the magnetic separation step may be incorporated as part of the assaying procedure. For instance, referring again to FIG. 1, a magnetic device (not shown) may be positioned adjacent to the medium 23 at a location at or near (e.g., downstream) the point of application, e.g., the sample pad 22. Thus, when the incubation mixture flows through the medium 23, any magnetic substances (released magnetic substances, partially cleaved reactive complexes, and/or unreacted complexes) become immobilized within a separation zone. The magnetic device may also be positioned upstream from a point in which conjugated probes are optionally contacted with the released reporters (e.g., a conjugate pad). The reporters, having been separated from the magnetic substances, may then be assayed using detection zones 31 and/or 35 as described above.

Description of Disclosure:
[0069] Triarylmethane dyes, for example, may have the following general structure: wherein R, R', and R" are independently selected from substituted and unsubstituted aryl groups, such as phenyl, naphthyl, anthracenyl, etc. The aryl groups may, for example, be substituted with functional groups, such as amino, hydroxyl, carbonyl, carboxyl, sulfonic, alkyl, and/or other known functional groups. When contacted with the dye, the amino group of the amine (e.g., ammonia, diamines, and/or tertiary amines) reacts with the central carbon atom of the dye. The addition of the amino group causes the dye to undergo a change in color. An example of the resulting structure is set forth below:

Description of Disclosure:
[0074] As indicated above, diarylmethanes may also be used in the present invention. One example of such a diarylmethane is 4,4'-bis (dimethylamino) benzhydrol (also known as "Michler's hydrol"), which has the following structure:

Description of Disclosure:
[0075] Still other examples include analogs of Michler's hydrol, such as Michler's hydrol leucobenzotriazole, Michler's hydrol leucomorpholine, Michier's hydrol leucobenzenesulfonamide, and so forth, as well as other diarylmethanes, such as malachite green leuco, malachite green carbinol, sodium 2,6-dichloroindopheno-late, rhodamine lactam, crystal violet lactone, and crystal violet leuco.

Description of Disclosure:
[0076] Generally speaking, any of a variety of devices may be utilized that employ that amine detection techniques of the present invention. For example, in one embodiment, an assay device may be utilized that incorporates an amine detection zone. Referring again to FIG. 1, for instance, the chromatographic medium 23 may define an amine detection zone 61 within which is contained a chemichromic dye. The amine detection zone 61 may generally be located downstream or upstream from the first enzyme detection zone 31 and/or the second enzyme detection zone 35. In the illustrated embodiment, for example, the amine detection zone 61 is located downstream from both the first enzyme detection zone 31 and the second enzyme detection zone 33. Although not required, this particular configuration may

help reduce the likelihood that any enzyme or enzyme inhibitors within the test sample inadvertently react with the chemichromic dye.

Description of Disclosure:
[0080] The amine detection zone 61 may generally provide any number of distinct detection regions so that a user may better determine the concentration of an amine within the test sample. Each region may contain the chemichromic dye, or may contain different dyes for reacting with different types of amines. For example, the amine detection zone 61 may include two or more distinct detection regions (e.g., lines, dots, etc.). The detection regions may be disposed in the form of lines in a direction substantially perpendicular to the flow of the test sample through the chromatographic medium 23. Likewise, in some embodiments, the detection regions may be disposed in the form of lines in a direction substantially parallel to the flow of the test sample through the assay device.

Description of Disclosure:
[0086] Because CIELAB color space is somewhat uniform, a single number may be calculated that represents the difference between two colors as perceived by a human. This difference is termed .DELTA.E and calculated by taking the square root of the sum of the squares of the three differences (.DELTA.L*, .DELTA.a*, and .DELTA.b*) between the two colors. In CIELAB color space, each .DELTA.E unit is approximately equal to a "just noticeable" difference between two colors. CIELAB is therefore a good measure for an objective device-independent color specification system that may be used as a reference color space for the purpose of color management and expression of changes in color. Using this test, color intensities (L*, a*, and b*) may thus be measured using, for instance, a handheld spectrophotometer from Minolta Co. Ltd. of Osaka, Japan (Model # CM2600d). This instrument utilizes the D/8 geometry conforming to CIE No.15, ISO 7724/1, ASTME1164 and JIS Z8722-1982 (diffused illumination/8-degree viewing system. The D65 light reflected by the specimen surface at an angle of 8 degrees to the normal of the surface is received by the specimen-measuring optical system. Still other suitable devices for measuring the intensity of a visual color may also be used in the present invention. For example, a suitable reflectance spectrophotometer or reader that may be used in the present invention is described in U.S. Patent App. Pub. No. 2003/0119202 to Kaylor, et al., which is incorporated herein in its entirety by reference thereto for all purposes.

Description of Disclosure:
[0091] The present invention provides a relatively simple, compact and cost-efficient device for accurately detecting enzymes (or enzyme inhibitors) and/or amines within a test sample (e.g., vaginal fluid). In this manner, for example, vaginal fluid may be tested in a single step for the presence of amines and also for the presence of other diseases or disorders. The test result may be visible so that it is readily observed by the person performing the test in a prompt manner and under test conditions conducive to highly reliable and consistent test results. The device may then be discarded as a unit when the test is concluded. Such a single step detection technique has a variety of benefits. For example, as discussed above, the detection of amines within a vaginal fluid test sample may indicate the presence of certain types of vaginal infection (e.g., bacterial vaginosis or trichomonas vaginitis), while the detection of an enzyme or enzyme inhibitor within the test sample may indicate the presence of other types of vaginal infection (e.g., candidal vaginitis).

Description of Disclosure:
[0096] The ability to form a membrane-based device for amine and/or enzyme assays was demonstrated. Initially, Millipore HF12002 porous nitrocellulose membranes were laminated onto corresponding supporting cards having a length of approximately 30 centimeters. Streptavidin (1.0 milligram per milliliter, Sigma-Aldrich Chemical Co., Inc.) was striped onto the membrane to form a first enzyme detection zone and Goldline.TM. (a polylysine solution obtained from British Biocell International) was striped onto the membrane (downstream from the first enzyme detection zone) to form a second enzyme

detection zone. Alpha-naphtholbenzein (ANB) (5 milligrams per milliliter, Sigma-Aldrich Chemical Co., Inc.) was also striped onto the membrane (downstream from the enzyme detection zones) to form an amine detection zone. The membrane was dried for 1 hour at 37.degree. C. A cellulosic fiber wicking pad (Millipore Co.) was attached to the end of the membrane closest to the amine detection zone. The assembled card was then cut into 4-millimeter wide devices. The resulting devices were sealed in a bag for storage.

Description of Disclosure:
[0097] The ability to form a membrane-based device for amine and/or enzyme assays was demonstrated. Initially, Millipore HF12002 porous nitrocellulose membranes were laminated onto corresponding supporting cards having a length of approximately 30 centimeters. Anti-biotin antibody (2.0 milligrams per milliliter, Sigma-Aldrich Chemical Co., Inc.) was striped onto the membrane to form a first enzyme detection zone and Goldline.TM. (a polylysine solution obtained from British Biocell International) was striped onto the membrane (downstream from the first enzyme detection zone) to form a second enzyme detection zone. ANB dye (Sigma-Aldrich Chemical Co., Inc.) was also striped onto the membrane (downstream from the enzyme detection zones) to form an amine detection zone. The membrane was dried for 1 hour at 37.degree. C. A cellulosic fiber wicking pad (Millipore Co.) was attached to the end of the membrane closest to the ANB dye zone. The assembled card was then cut into 4-millimeter wide devices. The resulting devices were sealed in a bag for storage.

Description of Disclosure:
[0099] Each sample was then transferred to a well present on a microtiter plate. The assay device samples of Example 2 were then inserted into each respective well to initiate the test. After allowing the assay to develop for 10 minutes, the color interisity of each detection zone was observed. The qualitative results are set forth below in Table 1. TABLE-US-00001 TABLE 1 Qualitative Color Results for Detection Zones First Enzyme Second Enzyme Amine Sample Detection Zone Detection Zone Detection Zone 1 Weak Strong Gray 2 None Strong Yellow 3 Strong None Gray 4 Strong Medium Gray

Description of Disclosure:
[0102] Each sample was then transferred to a well present on a microtiter plate. The assay device samples of Example 3 were then inserted into each respective well to initiate the test. After allowing the assay to develop for 10 minutes, the color intensity of each detection zone was observed. The qualitative results are set forth below in Table 2. TABLE-US-00002 TABLE 2 Qualitative Color Results for Detection Zones First Enzyme Second Enzyme Amine Sample Detection Zone Detection Zone Detection Zone 1 Strong None Yellow 2 Strong None Yellow 3 Strong Weak Yellow/Gray 4 Strong Medium Yellow/Gray 5 Medium Medium Yellow/gray 6 Medium Strong Yellow/gray 7 Weak Strong Gray 8 None Strong Gray

Description of Disclosure:
[0105] Each sample was then transferred to a well present on a microtiter plate. The assay device samples of Example 2 were then inserted into each respective well to initiate the test. After allowing the assay to develop for 10 minutes, the reflectance intensity of each detection zone was measured using a reflectance reader. The quantitative results are set forth below in Table 3. TABLE-US-00003 TABLE 3 Quantitative Color Intensity for Detection Zones Reflectance Reflectance Intensity (I.sub.1) Intensity (I.sub.2) Amine of First of Second Detection Sample Detection Zone Detection Zone Zone 1 2.0490 0.0263 0.2687 2 1.4070 0.6988 0.2890 3 1.1980 1.0920 0.2918 4 1.1080 1.3320 0.3169 5 0.8213 1.2800 0.3696 6 0.4298 1.2140 0.7020

CLAIMS:

11. A diagnostic test kit as defined in claim 10, further comprising a magnetic device positioned adjacent to said chromatographic medium to immobilize said magnetic particle within a separation zone.

Previous Doc Next Doc Go to Doc#

US 20050191704A1

(19) United States

(12) Patent Application Publication (10) Pub. No.: US 2005/0191704 A1

Boga et al. (43) Pub. Date: Sep. 1, 2005

(54) ASSAY DEVICES UTILIZING CHEMICHROMIC DYES

(75) Inventors: **RameshBabu Boga**, Roswell, GA (US); **John Gavin MacDonald**, Decatur, GA (US)

Correspondence Address:
DORITY & MANNING, P.A.
POST OFFICE BOX 1449
GREENVILLE, SC 29602-1449 (US)

(73) Assignee: **Kimberly-Clark Worldwide, Inc.**

(21) Appl. No.: 10/790,617

(22) Filed: Mar. 1, 2004

Publication Classification

(51) Int. Cl.[7] G01N 33/53; C12Q 1/04; G01N 33/543

(52) U.S. Cl. 435/7.1; 435/34

(57) **ABSTRACT**

An assay device for detecting amines within a test sample (e.g., vaginal fluid) is provided. The assay device comprises a detection zone within which a chemichromic dye is contained. The chemichromic dye is capable of undergoing a color change upon exposure to one or more amines within the test sample.

L48: Entry 4 of 87 File: PGPB Sep 1, 2005

DOCUMENT-IDENTIFIER: US 20050191704 A1
TITLE: Assay devices utilizing chemichromic dyes

Abstract Paragraph:
An assay device for detecting amines within a test sample (e.g., vaginal fluid) is provided. The assay device comprises a detection zone within which a chemichromic dye is contained. The chemichromic dye is capable of undergoing a color change upon exposure to one or more amines within the test sample.

Summary of Invention Paragraph:
[0005] In accordance with one embodiment of the present invention, an assay device for detecting the presence or absence of amines within a test sample is disclosed. The assay device comprises a fluidic medium (e.g., porous membrane, a flow channel, etc.) that defines a detection zone. Contained within the detection zone is a chemichromic dye that is capable of undergoing a detectable color change upon reaction with one or more amines. One particular example of a suitable chemichromic dye is an arylmethane, such as a diarylmethane or triarylmethane. In one embodiment, for example, the chemichromic dye is a triarylmethane having the following general structure: 1

Summary of Invention Paragraph:
[0006] wherein R, R', and R" are independently selected from substituted and unsubstituted aryl groups. The aryl groups may be, for example, phenyl groups, naphthyl groups, or anthracenyl groups, and may be amino-substituted, hydroxyl-substituted, carboxyl-substituted, alkyl-substituted, sulfonic-substituted, carbonyl-substituted, or combinations thereof. Specific examples of such triarylmethanes include, but are not limited to, pararosanilin, alpha-naphtholbenzein, naphthocrome green, or analogs thereof. As stated, other arylmethanes are also suitable for use in the present invention. For example, in one embodiment, the chemichromic dye is a diarylmethane, such as 4,4'-bis (dimethylamino) benzhydrol or analogs thereof.

Summary of Invention Paragraph:
[0007] In some cases, the assay device is also capable of detecting the presence or absence of an analyte within the test sample. For example, the fluidic medium is in fluid communication with detection probes that are optionally conjugated with a specific binding member for the analyte. In addition, the fluidic medium may also define a second detection zone within which a capture reagent is immobilized. The capture reagent is configured to bind to the detection probes or complexes thereof to generate a detection signal, wherein the amount of an analyte in the test sample is proportional to the intensity of the detection signal.

Summary of Invention Paragraph:
[0008] In accordance with another embodiment of the present invention, an assay device for detecting the presence or absence of both amines and an analyte within a test sample is disclosed. The assay device comprises a porous membrane that is in fluid communication with detection probes conjugated with a specific binding for the analyte. The porous membrane defines a first detection zone within which a triarylmethane dye is immobilized. The triarylmethane dye is capable of undergoing a detectable color change upon reaction with one or more amines. The porous

membrane also defines a second detection zone within a capture reagent is immobilized. The capture reagent is configured to bind to the detection probes or complexes thereof to generate a detection signal. The amount of an analyte in the test sample is proportional to the intensity of the detection signal.

Summary of Invention Paragraph:
[0009] In accordance with still another embodiment of the present invention, a method for detecting the presence or absence of amines within a test sample is disclosed. The method comprises contacting an assay device with a test sample containing one or more amines. The assay device comprises a fluidic medium that defines a detection zone, wherein a chemichromic dye is contained within the detection zone that undergoes a color change upon reacting with the amines. The method further comprises measuring the color intensity of the chemichromic dye at the detection zone after reacting with the amines, wherein the color intensity corresponds to a certain concentration of the amines within the test sample. In some cases, this color intensity may also be compared to the color intensity of a chemichromic dye that is not reacted with amines. This dye, which is not reacted with amines, may be contained within a control zone defined by the fluidic medium.

Brief Description of Drawings Paragraph:
[0012] FIG. 1 is a perspective view of one embodiment of a flow-through assay device of the present invention;

Brief Description of Drawings Paragraph:
[0013] FIG. 2 is a perspective view of another embodiment of a flow-through assay device of the present invention;

Detail Description Paragraph:
[0023] In general, the present invention is directed to an assay device for detecting the presence of amines (monoamines, diamines, and/or tertiary amines) in a test sample. Specifically, the assay device includes a detection zone within which is contained a chemichromic dye, i.e., a dye that exhibits a detectable color change upon chemical reaction with one or more functional groups, such as amino groups. The assay device is also multi-functional in that it is capable of simultaneously detecting the presence of an analyte within the test sample.

Detail Description Paragraph:
[0024] Referring to FIG. 1, for instance, one embodiment of a membrane-based flow-through assay device 20 that may be formed according to the present invention will now be described in more detail. As shown, the device 20 contains a porous membrane 23 that acts as a fluidic medium and is optionally supported by a rigid material 21. In general, the porous membrane 23 may be made from any of a variety of materials through which the test sample is capable of passing. For example, the materials used to form the porous membrane 23 may include, but are not limited to, natural, synthetic, or naturally occurring materials that are synthetically modified, such as polysaccharides (e.g., cellulose materials such as paper and cellulose derivatives, such as cellulose acetate and nitrocellulose); polyether sulfone; polyethylene; nylon; polyvinylidene fluoride (PVDF); polyester; polypropylene; silica; inorganic materials, such as deactivated alumina, diatomaceous earth, MgSO.sub.4, or other inorganic finely divided material uniformly dispersed in a porous polymer matrix, with polymers such as vinyl chloride, vinyl chloride-propylene copolymer, and vinyl chloride-vinyl acetate copolymer; cloth, both naturally occurring (e.g., cotton) and synthetic (e.g., nylon or rayon); porous gels, such as silica gel, agarose, dextran, and gelatin; polymeric films, such as polyacrylamide; and so forth. In one particular embodiment, the porous membrane 23 is formed from nitrocellulose and/or polyether sulfone materials. It should be understood that the term "nitrocellulose" refers to nitric acid esters of cellulose, which may be nitrocellulose alone, or a mixed ester of nitric acid and other acids, such as aliphatic carboxylic acids having from 1 to 7 carbon atoms.

Detail Description Paragraph:
[0025] The device 20 may also contain an absorbent pad 28. The absorbent pad 28 generally receives fluid that has migrated through the entire porous membrane 23. As is well known in the art, the absorbent pad 28 may assist in promoting capillary action and fluid flow through the membrane 23.

Detail Description Paragraph:
[0030] wherein R, R', and R" are independently selected from substituted and unsubstituted aryl groups, such as phenyl, naphthyl, anthracenyl, etc. The aryl groups may, for example, be substituted with functional groups, such as amino, hydroxyl, carbonyl, carboxyl, sulfonic, alkyl, and/or other known functional groups. When contacted with the dye, the amino group of the amine (e.g., ammonia, diamines, and/or tertiary amines) reacts with the central carbon atom of the dye. The addition of the amino group causes the dye to undergo a change in color. An example of the resulting structure is set forth below: 3

Detail Description Paragraph:
[0035] As indicated above, diarylmethanes may also be used in the present invention. One example of such a diarylmethane is 4,4'-bis (dimethylamino) benzhydrol (also known as "Michler's hydrol"), which has the following structure: 8

Detail Description Paragraph:
[0036] Still other examples include analogs of Michler's hydrol, such as Michler's hydrol leucobenzotriazole, Michler's hydrol leucomorpholine, Michler's hydrol leucobenzenesulfonamide, and so forth, as well as other diarylmethanes, such as malachite green leuco, malachite green carbinol, sodium 2,6-dichloroindopheno-late, rhodamine lactam, crystal violet lactone, and crystal violet leuco.

Detail Description Paragraph:
[0038] In some cases, the chemichromic dye is applied in a manner so that it does not substantially diffuse through the matrix of the porous membrane 23. This enables a user to readily detect the change in color that occurs upon reaction of the dye with an amine. For instance, the chemichromic dye may form an ionic and/or covalent bond with functional groups present on the surface of the porous membrane 23 so that it remains immobilized thereon. For example, in one embodiment, a positively-charged chemichromic dye may form an ionic bond with negatively-charged carboxyl groups present on the surface of some porous membranes (e.g., nitrocellulose). In other embodiments, the use of particles may facilitate the immobilization of the chemichromic dye at the detection zone 31. Namely, the dye may be coated onto particles (sometimes referred to as "beads" or "microbeads") that are then immobilized on the porous membrane 23 of the assay device 20. In this manner, the dye is able to readily contact a test sample flowing through the membrane 23. For instance, naturally occurring particles, such as nuclei, mycoplasma, plasmids, plastids, mammalian cells (e.g., erythrocyte ghosts), unicellular microorganisms (e.g., bacteria), polysaccharides (e.g., agarose), and so forth, may be used. Further, synthetic particles may also be utilized. For example, in one embodiment, latex particles may be labeled with the chemichromic dye. Although any latex particle may be used in the present invention, the latex particles are typically formed from polystyrene, butadiene styrenes, styreneacrylic-vinyl terpolymer, polymethylmethacrylate, polyethylmethacrylate, styrene-maleic anhydride copolymer, polyvinyl acetate, polyvinylpyridine, polydivinylbenzene, polybutyleneterephthalate- , acrylonitrile, vinylchloride-acrylates, and so forth, or an aldehyde, carboxyl, amino, hydroxyl, or hydrazide derivative thereof. Other suitable particles may be described in U.S. Pat. No. 5,670,381 to Jou, et al. and U.S. Pat. No. 5,252,459 to Tarcha, et al., which are incorporated herein in their entirety by reference thereto for all purposes.

Detail Description Paragraph:

[0041] The detection zone 31 may generally provide any number of distinct detection regions so that a user may better determine the concentration of a particular analyte within a test sample. Each region may contain the chemichromic dye, or may contain different dyes for reacting with different types of amines. For example, the detection zone 31 may include two or more distinct detection regions (e.g., lines, dots, etc.). The detection regions may be disposed in the form of lines in a direction that is substantially perpendicular to the flow of the test sample through the assay device 20. Likewise, in some embodiments, the detection regions may be disposed in the form of lines in a direction that is substantially parallel to the flow of the test sample through the assay device.

Detail Description Paragraph:
[0042] If desired, the assay device 20 may employ a control zone 32 that is applied with the same chemichromic dye applied to the detection zone 31 and positioned downstream from the detection zone 31. In addition, the detection zone 31 may be provided with an amount of dye that is equal to or in excess of the amount needed to fully react with all of the amines present within the test sample. Thus, amines from the test sample will react only at the detection zone 31 and not at the control zone 32. In this manner, the color of the control zone 32 will generally remain unchanged so that it may be compared to the color of the detection zone 31 for determining the extent to which it changed after reaction with the amines. Similar to the detection zone 31, the control zone 32 may also provide any number of distinct regions:

Detail Description Paragraph:
[0048] Because CIELAB color space is somewhat uniform, a single number may be calculated that represents the difference between two colors as perceived by a human. This difference is termed .DELTA.E and calculated by taking the square root of the sum of the squares of the three differences (.DELTA.L*, .DELTA.a*, and .DELTA.b*) between the two colors. In CIELAB color space, each .DELTA.E unit is approximately equal to a "just noticeable" difference between two colors. CIELAB is therefore a good measure for an objective device-independent color specification system that may be used as a reference color space for the purpose of color management and expression of changes in color. Using this test, color intensities (L*, a*, and b*) may thus be measured using, for instance, a handheld spectrophotometer from Minolta Co. Ltd. of Osaka, Japan (Model # CM2600d). This instrument utilizes the D/8 geometry conforming to CIE No. 15, ISO 7724/1, ASTME1164 and JIS Z8722-1982 (diffused illumination/8-degree viewing system. The D65 light reflected by the specimen surface at an angle of 8 degrees to the normal of the surface is received by the specimen-measuring optical system. Still other suitable devices for measuring the intensity of a visual color may also be used in the present invention. For example, one suitable reflectance reader is described in U.S. Patent App. Pub. No. 2003/0119202 to Kaylor, et al., which is incorporated herein in its entirety by reference thereto for all purposes.

Detail Description Paragraph:
[0050] Besides detecting the presence of amines in a test sample, the assay device of the present invention is also able to detect the presence of an analyte. In this manner, for example, vaginal fluid may be simultaneously tested for the presence of amines and also for the presence of other diseases or disorders. Referring to FIG. 2, for example, one embodiment of an assay device 120 is shown that is configured to simultaneously detect the presence of an analyte and amines within a test sample. Similar to the assay device 20 of FIG. 1, the assay device 120 contains a porous membrane 123 optionally supported by a rigid material 121. The assay device 120 also contains a sampling pad (not shown), a conjugate pad 122, and an absorbent pad 128 in fluid communication with the porous membrane 123.

Detail Description Paragraph:
[0051] To facilitate accurate detection of an analyte within the test sample, a predetermined amount of detection probes may be applied at various locations of the

device 120, such as to a conjugate pad 122. Any substance generally capable of producing a signal that is detectable visually or by an instrumental device may be used as detection probes. Various suitable substances may include calorimetric or fluorescent chromogens; catalysts; luminescent compounds (e.g., fluorescent, phosphorescent, etc.); radioactive compounds; visual labels, including colloidal metallic (e.g., gold) and non-metallic particles, dyed particles, hollow particles, enzymes or substrates, or organic polymer latex particles; liposomes or other vesicles containing signal producing substances; and so forth. For instance, some enzymes suitable for use as detection probes are disclosed in U.S. Pat. No. 4,275,149 to Litman. et al., which is incorporated herein in its entirety by reference thereto for all purposes. One example of an enzyme/substrate system is the enzyme alkaline phosphatase and the substrate nitro blue tetrazolium-5-bromo-4-c- hloro-3-indolyl phosphate, or derivative or analog thereof, or the substrate 4-methylumbelliferyl-phosphate. In an alternative probe system, the detection probes may be a fluorescent compound, such as fluorescein, phycobiliprotein, rhodamine and their derivatives and analogs. Other suitable detection probes may be described in U.S. Pat. No. 5,670,381 to Jou, et al. and U.S. Pat. No. 5,252,459 to Tarcha. et al., which are incorporated herein in their entirety by reference thereto for all purposes.

Detail Description Paragraph:
[0057] Similar to the detection zone 131, the detection zone 135 may also provide any number of distinct detection regions so that a user may better determine the concentration of a particular analyte within a test sample. Each region may contain the same capture reagents, or may contain different capture reagents for capturing multiple analytes. For example, the detection zone 135 may include two or more distinct detection regions (e.g., lines, dots, etc.). The detection regions may be disposed in the form of lines in a direction that is substantially perpendicular to the flow of the test sample through the assay device 120. Likewise, in some embodiments, the detection regions may be disposed in the form of lines in a direction that is substantially parallel to the flow of the test sample through the assay device.

Detail Description Paragraph:
[0058] Although the second detection zone 135 provides accurate results for detecting an analyte, it is sometimes difficult to determine the relative concentration of the analyte within the test sample under actual test conditions. Thus, the assay device 120 may also include a calibration zone 137. In this embodiment, the calibration zone 137 is formed on the porous membrane 123 and is positioned downstream from the second detection zone 135. Alternatively, however, the calibration zone 137 may also be positioned upstream from the detection zone 135. The calibration zone 137 may be provided with a capture reagent that is capable of binding to calibration probes or uncomplexed detection probes that pass through the length of the membrane 123. When utilized, the calibration probes may be formed from the same or different materials as the detection probes. Generally speaking, the calibration probes are selected in such a manner that they do not bind to the capture reagent at the detection zone 135.

Detail Description Paragraph:
[0059] The capture reagent of the calibration zone 137 may be the same or different than the capture reagent used in the detection zone 135. For example, in one embodiment, the capture reagent is a biological capture reagent. In addition, it may also be desired to utilize various non-biological materials for the capture reagent of the calibration zone 137. The polyelectrolytes may have a net positive or negative charge, as well as a net charge that is generally neutral. For instance, some suitable examples of polyelectrolytes having a net positive charge include, but are not limited to, polylysine (commercially available from Sigma-Aldrich Chemical Co., Inc. of St. Louis, Mo.), polyethyleneimine; epichlorohydrin-functionalized polyamines and/or polyamidoamines, such as poly(dimethylamine-co-epichlorohydrin); polydiallyldimethyl-ammonium chloride; cationic cellulose

derivatives, such as cellulose copolymers or cellulose derivatives grafted with a quaternary ammonium water-soluble monomer; and so forth. In one particular embodiment, CelQuat.RTM. SC-230M or H-100 (available from National Starch & Chemical, Inc.), which are cellulosic derivatives containing a quaternary ammonium water-soluble monomer, may be utilized. Moreover, some suitable examples of polyelectrolytes having a net negative charge include, but are not limited to, polyacrylic acids, such as poly(ethylene-co-methacrylic acid, sodium salt), and so forth. It should also be understood that other polyelectrolytes may also be utilized, such as amphiphilic polyelectrolytes (i.e., having polar and non-polar portions). For instance, some examples of suitable amphiphilic polyelectrolytes include, but are not limited to, poly(styryl-B-N-methyl 2-vinyl pyridinum iodide) and poly(styryl-b-acrylic acid), both of which are available from Polymer Source, Inc. of Dorval, Canada. Further examples of internal calibration systems that utilize polyelectrolytes are described in more detail in U.S. Patent App. Publication No. 2003/0124739 to Song, et al., which is incorporated herein in it entirety by reference thereto for all purposes.

Detail Description Paragraph:
[0061] Referring to FIG. 3, one embodiment of a method for simultaneously detecting the presence of amines and an analyte within a test sample using the assay device 120 will now be described in more detail. Initially, a test sample containing amines "A" and an analyte "B" is applied to the sample pad (not shown) and travels in the direction "L" to the conjugate pad 122, where the analyte B mixes with detection probes 141 conjugated with an antibody and calibration probes 143 (may or may not be conjugated). The analyte B binds with the conjugated detection probes 141 to form analyte/conjugated probe complexes 149. These complexes 149 travel on to the second detection zone 135 and bind to an antibody 153. Finally, the calibration probes 143 travel through both the detection zone 135 to bind with a polyelectrolyte (not shown) at the calibration zone 137. Once captured, the intensity of the signal of the detection probes 141 may be determined (visually or with instrumentation) at the second detection zone 135. In addition, the intensity of the signal of the calibration probes 143 may also be measured at the calibration zone 137. The absolute amount of the analyte may be ascertained by comparing the signal intensity at the detection zone 131 with the signal intensity at the calibration zone 137.

Detail Description Paragraph:
[0063] Although various assay device configuration have been described herein, it should be understood that any known assay device may be utilized that is capable of incorporating a chemichromic dye in accordance with the present invention. For example, besides flow-through devices that utilize a porous membrane as a fluidic medium, such as described above, an assay device that utilizes one or more fluidic channels as a fluidic medium for the test sample may also be used in the present invention. Likewise, other detection techniques may be used for determining the presence of an analyte within the test sample. For example, electrochemical affinity assays may also be utilized, which detect an electrochemical reaction between an analyte (or complex thereof) and a capture ligand on an electrode strip. For example, various electrochemical assays are described in U.S. Pat. No. 5,508,171 to Walling, et al.; U.S. Pat. No. 5,534,132 to Vreeke, et al.; U.S. Pat. No. 6,241,863 to Monbouguette; U.S. Pat. No. 6,270,637 to Crismore, et al.; U.S. Pat. No. 6,281,006 to Heller, et al.; and U.S. Pat. No. 6,461,496 to Feldman, et al., which are incorporated herein in their entirety by reference thereto for all purposes.

Detail Description Paragraph:
[0064] In addition, it should be understood that both sandwich and competitive assay formats may be used to detect an analyte according to the present invention. Techniques and configurations of sandwich and competitive assay formats are well known to those skilled in the art. For example, sandwich formats assay formats typically involve mixing the test sample with antibodies to the analyte. These

antibodies are mobile and linked to the label. This mixture is then contacted with a chromatographic medium containing a band or zone of immobilized antibodies to the analyte. The chromatographic medium is often in the form of a strip resembling a dipstick. When the complex of the analyte and the labeled antibody reaches the zone of the immobilized antibodies on the chromatographic medium, binding occurs and the bound labeled antibodies are localized at the zone. This indicates the presence of the analyte. This technique may be used to obtain quantitative or semi-quantitative results. Some examples of such sandwich-type assays are described by U.S. Pat. No. 4,168,146 to Grubb, et al. and U.S. Pat. No. 4,366,241 to Tom, et al., which are incorporated herein in their entirety by reference thereto for all purposes. In a competitive assay, the probe is generally a labeled analyte or analyte-analog that competes for binding of an antibody with any unlabeled analyte present in the sample. Competitive assays are typically used for detection of analytes such as haptens, each hapten being monovalent and capable of binding only one antibody molecule. Examples of competitive immunoassay devices are described in U.S. Pat. No. 4,235,601 to Deutsch, et al., U.S. Pat. No. 4,442,204 to Liotta, and U.S. Pat. No. 5,208,535 to Buechler, et al., which are incorporated herein in their entirety by reference thereto for all purposes. Various other device configurations and/or assay formats are also described in U.S. Pat. No. 5,395,754 to Lambotte, et al.; U.S. Pat. No. 5,670,381 to Jou, et al.; and U.S. Pat. No. 6,194,220 to Malick, et al., which are incorporated herein in their entirety by reference thereto for all purposes.

Detail Description Paragraph:
[0065] The present invention provides a relatively simple, compact and cost-efficient device for accurately detecting amines and optionally other analytes within a test sample (e.g., vaginal fluid). The test result may be visible so that it is readily observed by the person performing the test in a prompt manner and under test conditions conducive to highly reliable and consistent test results. The device may then be discarded as a unit when the test is concluded.

Detail Description Paragraph:
[0070] The ability to form a lateral flow assay device with multiple detection zones was demonstrated. Three sets of assay device samples were prepared (Samples 1-3). Each assay device sample was formed from a nitrocellulose porous membrane (HF 12002 from Millipore, Inc.) having a length of approximately 30 centimeters laminated onto a corresponding supporting card. Chemichromic detection zones were formed on each of the samples using two different stock solutions of alpha-naphtholbenzein (Sigma-Aldrich Chemical Company), each of which had a volume of 6200 microliters and contained a methanol/water solvent (4/6 ratio). The first stock solution contained alpha-naphtholbenzein in a concentration of 5.0 milligrams per milliliter, while the second stock solution contained alpha-naphtholbenzein in a concentration of 2.3 milligrams per milliliter. One (1) microliter of these stock solutions was then stripped onto the samples to form the chemichromic detection zones. In addition, monoclonal antibody for C-reactive protein (CRP Mab2) (A#5804, available from BiosPacific, Inc., concentration of 1 milligram per milliliter) was immobilized downstream from the chemichromic detection zone on the porous membrane to form the other detection zone. The samples were then dried for 1 hour at a temperature of 37.degree. C.

Detail Description Paragraph:
[0072] The ability to detect an amine using a lateral flow assay device was demonstrated. Assay device samples were prepared as described above in Example 4. Thereafter, solutions were provided that contained varying concentrations of putrescine (Sigma-Aldrich Chemical Company of Milwaukee, Wis., USA, 99% pure) in acetonitrile, i.e., 0.0, 0.15, 0.30, 0.60, 1.25, 2.50, 5.00, and 10.00 milligrams of putrescine per milliliter. These solutions were applied to the samples, and reflectance readings were then measured for the samples as shown in FIG. 7. As indicated, the dye readily detected the presence of putrescine. Further, the level of detection sensitivity was readily controlled by varying the dye concentration.

CLAIMS:

1. An assay device for detecting the presence or absence of amines within a test sample, said assay device comprising a fluidic medium that defines a detection zone, wherein a chemichromic dye is contained within said detection zone, said chemichromic dye being capable of undergoing a detectable color change upon reaction with one or more amines.

2. An assay device as defined in claim 1, wherein said chemichromic dye is an arylmethane.

3. An assay device as defined in claim 2, wherein said arylmethane is selected from the group consisting of diarylmethanes and triarylmethanes.

4. An assay device as defined in claim 2, wherein said chemichromic dye is a triarylmethane having the following general structure: 9wherein R, R', and R" are independently selected from substituted and unsubstituted aryl groups.

5. An assay device as defined in claim 4, wherein said aryl groups are phenyl groups, naphthyl groups, or anthracenyl groups.

6. An assay device as defined in claim 5, wherein at least one of said aryl groups is amino-substituted, hydroxyl-substituted, carboxyl-substituted, sulfonic-substituted, alkyl-substituted, carbonyl-substituted, or combinations thereof.

7. An assay device as defined in claim 4, wherein said triarylmethane is pararosanilin, alpha-naphtholbenzein, naphthocrome green, or analogs thereof.

8. An assay device as defined in claim 3, wherein said chemichromic dye is a diarylmethane.

9. An assay device as defined in claim 8, wherein said diarylmethane is 4,4'-bis (dimethylamino) benzhydrol or analogs thereof.

10. An assay device as defined in claim 1, wherein said fluidic medium is a porous membrane.

11. An assay device as defined in claim 1, wherein said fluidic medium includes at least one flow channel.

12. An assay device as defined in claim 1, wherein said fluidic medium is in fluid communication with detection probes.

13. An assay device as defined in claim 12, wherein said detection probes are conjugated with a specific binding member for the analyte.

14. An assay device as defined in claim 13, wherein said fluidic medium defines a second detection zone within which is immobilized a capture reagent, said capture reagent being configured to bind to said detection probes or complexes thereof to generate a detection signal, wherein the amount of an analyte in the test sample is proportional to the intensity of said detection signal.

15. An assay device as defined in claim 1, wherein said fluidic medium further defines a control zone within which a chemichromic dye is contained, said control zone being located downstream from said detection zone.

16. An assay device for detecting the presence or absence of both amines and an analyte within a test sample, said assay device comprising a porous membrane that is in fluid communication with detection probes conjugated with a specific binding

for the analyte, said porous membrane defining: a first detection zone within which a triarylmethane dye is immobilized, said triarylmethane dye being capable of undergoing a detectable color change upon reaction with one or more amines; and a second detection zone within which a capture reagent is immobilized, said capture reagent being configured to bind to said detection probes or complexes thereof to generate a detection signal, wherein the amount of an analyte in the test sample is proportional to the intensity of said detection signal.

17. An assay device as defined in claim 16, wherein said triarylmethane has the following general structure: 10wherein R, R', and R" are independently selected from substituted and unsubstituted aryl groups.

18. An assay device as defined in claim 17, wherein said aryl groups are phenyl groups, naphthyl groups, or anthracenyl groups.

19. An assay device as defined in claim 18, wherein at least one of said aryl groups is amino-substituted, hydroxyl-substituted, carboxyl-substituted, alkyl-substituted, sulfonic-substituted, carbonyl-substituted, or combinations thereof.

20. An assay device as defined in claim 16, wherein said triarylmethane is pararosanilin, alpha-naphtholbenzein, naphthocrome green, or analogs thereof.

21. An assay device as defined in claim 16, wherein said porous membrane further defines a control zone within which a chemichromic dye is contained, said control zone being located downstream from said detection zone.

22. A method for detecting the presence or absence of amines within a test sample, said method comprising: i) contacting an assay device with a test sample containing one or more amines, said assay device comprising a fluidic medium that defines a detection zone, wherein a chemichromic dye is contained within said detection zone that undergoes a color change upon reacting with said amines; and ii) measuring the color intensity of said chemichromic dye at said detection zone after reacting with said amines, wherein said color intensity corresponds to a certain concentration of said amines within the test sample.

Previous Doc Next Doc Go to Doc#